Not a fairy tale: Archives tell a story about clammed up natives and crabby invaders

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The Walrus and the Carpenter speaking to the Oysters, as portrayed by illustrator John Tenniel

'Herrlicher als der bestirnte Himmel über uns, ist nur das Universum der Logik in uns!'

Professor Abronsius, *Tanz der Vampire*

Eidesstattliche Erklärung

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Sarah Hayer

CHRISTIAN-ALBRECHTS-UNIVERSITY KIEL

Abstract

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Doctor of Biology

Not a fairy tale: Archives tell a story about clammed up natives and crabby invadors

by Sarah HAYER

Natural history museum collections are valuable archives of the past. They store selective moments in form of individual organisms that are linked to a particular date and place. Repeated collection events over several years of identical species from the same places results in time series. These time series are invaluable because they store information about the changes in biodiversity and adaptations of species. By applying modern analyses techniques like ancient DNA (aDNA) methods and shotgun sequencing, the genetic data of worldwide 1.5 billion historic collection entries can be made available and investigated today. Because of this research potential, natural history museum collections represent an essential basis for scientific questions, particularly in relation to long-term environmental changes caused by climate change and globalisation.

In the course of this thesis, three studies have been conducted on the basis of museum collections in the background of climate change and globalisation. First, the studies aimed to investigate the extinction event of the native European flat oyster *Ostrea edulis*, as well as the impact of the neozoan common limpet slipper *Crepidula fornicata* on the oyster, since it was soon after its introduction accused to displace *O. edulis*. Secondly, the historical phylogeography of *O. edulis* was reconstructed to enlighten the processes of the extinction event further. Thirdly, the genetic population structure of long-established neozoa like the Chinese mitten crab *Eriocheir sinensis* was investigated in order to find potential adaptation or differentiation processes to

its new environment. Both native and neozoan species were selected for their ecological and economic importance, which is why many museum collections hold time series of these species.

The results of these studies showed that the extinction event of *O. edulis* in the North Sea can be successfully traced based on collection material. The shallow oyster beds on the coasts went extinct first, whereas the last living individual of the deeper oyster beds in the central North Sea was found in 1939. Moreover, the reconstruction of the historical distribution revealed that the population of *C. fornicata* increased only after the oyster beds went extinct. Thus, *C. fornicata* is despite its bad reputation not to blame for the extinction event of the European flat oyster in the North Sea.

Additionally, by using aDNA methods on dry shells from museum collections, the historical phylogeography of *O. edulis* was successfully depicted in its native range for the first time. These modern methods unveiled the historical population structure of the European flat oyster across Europe in the late 19^{th} century – including the now extinct populations of the Wadden Sea. The innovation of this historical study in comparison with present-day studies was the discovery of the autochthonous haplogroup in the Wadden Sea. This haplogroup has not yet been detected in current oyster beds in other locations, assuming this haplogroup could be extinct today. This offers a possible explanation why the oyster has not resettled the Wadden Sea until now.

Lastly, the public databases (e.g. GenBank, BOLD) were used for population genetic analyses on the Chinese mitten crab *E. sinensis*. This invasive crab has been introduced to Europe at the beginning of the 20^{th} century and spread rapidly over countries ever since. The results revealed unique haplotypes occurring in Northern Germany that has not been found in the native range. These haplotypes suggest genetic differentiation and adaptation processes in response to the new environment during the past century.

Finally, the natural history collections proved to be a valuable tool for different research questions. Whereas the impact of climate change could not be verified on the basis of these preliminary studies, the influence of globalisation is evident. Therefore the results of the historical studies will help to better understand the processes in the future.

CHRISTIAN-ALBRECHTS-UNIVERSITÄT ZU KIEL

Zusammenfassung

Mathematisch-Naturwissenschaftliche Fakultät
Zoologisches Museum Kiel

Doktor der Biologie

Not a fairy tale:
Archives tell a story about clammed up natives and crabby invadors

von Sarah HAYER

Die Sammlungen der Naturkundemuseen sind wertvolle Archive der Vergangenheit. Sie bewahren einzelne Momente in Form von individuellen Organismen, die mit einem bestimmten Datum und einem Ort verknüpft sind. Durch wiederholte Sammlungsereignisse über mehrere Jahre hinweg von denselben Arten aus den gleichen Orten entstehen Zeitreihen in den musealen Sammlungen. Diese Zeitreihen sind von unschätzbaren Wert, da sie Informationen über die Veränderungen in der Biodiversität und Anpassungen von Arten beinhalten. Durch die Anwendung von modernen Analyseverfahren wie ancient DNA (aDNA) und Shotgun-Sequencing Methoden, können die genetischen Informationen der weltweit über 1,5 Milliarden Sammlungseinträge heute zugänglich gemacht und untersucht werden. Aufgrund dieses Forschungspotentials stellen die Sammlungen der Naturkundemuseen eine grundlegende Basis für viele Wissenschaftsfragen dar, vor allem in Bezug auf Langzeitveränderungen in der Umwelt durch den Einfluss des Klimawandels und der Globalisierung.

Im Zuge dieser Doktorarbeit wurde drei Studien auf der Basis der musealen Sammlungen und im Hintergrund von Klimawandel und Globalisierung durchgeführt. Zunächst wurde das Aussterbeereignis der heimischen Europäischen Auster *Ostrea edulis* untersucht, sowie die Auswirkungen der Pantoffelschnecke *Crepidula fornicata*, einer Neozoe, welche seit ihrer Einschleppung am Aussterben der Auster verantwortlich gemacht wird. Zweitens wurde die historische Phylogeographie

der *O. edulis* rekonstruiert, um den Prozess des Aussterbeereignisses noch weiter zu studieren. Drittens wurde die genetische Populationsstruktur einer lang etablierten neozoischen Art wie der Chinesischen Wollhandkrabbe *Eriocheir sinensis* untersucht, um potentielle Anpassungs- und Differenzierungsprozesse an die neue Umgebung zu untersuchen. Sowohl heimische als auch neozoische Arten wurden aufgrund ihrer ökologischen und ökonomischen Bedeutung ausgewählt, weshalb auch viele Museen über Zeitreihen dieser Arten verfügen.

Die Ergebnisse dieser Studien zeigen, dass anhand der Sammlungen das Aussterbeereignis der *O. edulis* in der Nordsee erfolgreich nachvollzogen werden kann. Die Austernbanken in den flachen Küstengebieten starben zuerst aus, wohingegen die letzte lebende Auster der tieferen Austernbänke in der zentralen Nordsee im Jahre 1939 gefunden wurde. Zusätzlich ergab die Rekonstruktion der historischen Ausbreitung, dass die Populationen der *C. fornicata* erst nach dem Aussterbeereignis der Auster zugenommen haben. Daher ist *C. fornicata*, entgegen ihres schlechten Rufs, nicht für das Aussterbeereignis der Europäischen Auster verantwortlich.

Darüber hinaus wurde die historische Phylogeographie der *O. edulis* zum ersten Mal unter Verwendung von aDNA Methoden an trockenen Austernschalen aus den musealen Sammlungen erfolgreich für den heimischen Raum dargestellt. Diese modernen Methoden enthüllen die historische Populationsstruktur der Europäischen Auster gegen Ende des 19. Jahrhunderts über den gesamten Europäischen Raum – inklusive des Wattenmeeres, wo die Auster heute ausgestorben ist. Der Vorteil dieser Studie mit historischen Tieren gegenüber modernen Untersuchungen ist die Entdeckung der autochthonen Haplogruppe im Wattenmeer. Diese Haplogruppe ist bis heute nicht an anderen Orten gefunden worden, daher besteht die Annahme, dass sie ausgestorben sein könnte. Dies würde erklären, warum die Auster bis heute nicht in das Wattenmeer zurückgekehrt ist.

Für die letzte Studie wurden öffentliche Datenbanken (GenBank, BOLD) genutzt, um populationsgenetische Analysen über die Chinesische Wollhandkrabbe *E. sinensis* durchzuführen. Diese invasive Art wurde zu Beginn des 20. Jahrhunderts in Europa eingeschleppt, wo sie sich rasch über mehrere Länder ausbreitete. Die Ergebnisse präsentieren einzigartige Haplotypen, die in Norddeutschland vorkommen und bisher nicht im heimischen Raum gefunden worden sind. Diese Haplotypen lassen vermuten, dass genetische Anpassungs- und Differenzierungsprozesse an die neue Umgebung während des letzten Jahrhunderts stattgefunden haben.

Letztlich ist bewiesen worden, dass die Sammlungen der Naturkundemuseen eine wertvolle Ressource für unterschiedliche wissenschaftliche Fragestellungen darstellen. Obwohl der Einfluss des Klimawandels auf die Veränderungen der Arten und Ökosysteme anhand dieser vorläufigen Studien nicht nachgewiesen werden konnte, liegt der Einfluss der Globalisierung auf der Hand. Aufgrund dieser Ergeb-

nisse werden Studien über vergangene Ereignisse helfen die Prozesse in der Zukunft besser zu verstehen.

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Contents

Introduction

Global change and its consequences for marine life

We live in a world where the lasting changes in oceans are severe. Two major factors are causing the change: climate change and globalization, which are both man-made. Since the beginning of the Industrial Revolution, humans have burned huge amounts of fossil fuels. In response to that, the planet's atmosphere has been warmed up by 1.14°C since the late 19th century ('NASA: Global Climate Change', n.d.). The IPCC (Stocker et al., 2013) revealed that the oceans had absorbed more than 93% of the excess heat from man-made emissions in the past 50 years (Laffoley and Baxter, 2016). This causes the ocean temperatures to rise by approximately 0.13°C per decade since the late 19th century (Laffoley and Baxter, 2016; NOAA, 2020). Increasing ocean temperatures affect marine species and ecosystems deeply. One prominent example of an endangered ecosystem is the Great Barrier Reef off Australia. The largest marine park in the world is made up of more than 2 900 separate coral reefs. The Great Barrier Reef is a highly diverse ecosystem that harbours about 1 500 fish species, more than 4 000 mollusc species, 500 algae species, six of the world's seven marine turtle species, 24 seabird species, more than 30 whale species and the dugong (Johnson et al., 2007). Since 1998, the rising temperatures caused four massive coral bleaching events, leading to a decline of live corals of up to 51% (Stuart-Smith et al., 2018). The latest, severe coral bleaching event took place in 2020, affecting all three regions of the Great Barrier Reef for the first time – an area the size of the United Kingdom, the Netherlands and Switzerland combined – with presumably dramatic consequences for the ecosystem ('Climate change triggers Great Barrier Reef bleaching published 7 April 2020', n.d.).

The increasing ocean temperatures led also to a loss of breeding grounds and distribution changes as species search for more favourable environmental conditions. For example, the distribution range of the Atlantic cod *Gardus morhua* is altered by the warming temperatures. This species belongs to the most important commercial fish species internationally (Mieszkowska et al., 2009). Beaugrand and Kirby, 2010 investigated the correlation between the climate change and the distribution change of the Atlantic cod in the North Sea over the past 100 years, showing that

distribution shifts of the cod to the north are amongst others a consequence of the distribution change of the plankton due to warmer temperatures (Beaugrand and Kirby, 2010). The copepod *Calanus fimmarchicus*, which is an important prey of cod larvae, is found less frequently in the North Sea, which explains the strong reduction in cod recruitment and biomass observed in the North Sea (Beaugrand et al., 2003). Thus, climate change is currently moving the boundary between the polar and temperate biome northwards with huge effects for biodiversity and the functioning of ecosystems (Beaugrand et al., 2008).

Distributional changes in marine organisms occur not only due to climate change, but also due to globalization (Galil et al., 2008; Geburzi et al., 2015). Since the Industrialization, shipping traffic has increased extremely and is still increasing. This is evident in the port of Hamburg alone, where freight traffic has increased by tremendous 91% per year on average since the middle of the 19^{th} century (Nord, 2019). With globalization, not only the traffic increased, but new channels were built in the last centuries – like the Suez canal or the Kiel canal for example. The intention in building the canals was clearly an economic one, as areas that were previously clearly separated became connected to each other in order to reduce both the travel distance and the travel time to a minimum. All ecological consequences, however, were neglected at that time and only recently came into focus of science.

International shipping traffic not only transports economical goods across the oceans, but neozoa as well. Neozoa are defined as animal species, that were introduced directly or unintentionally by man to a certain area after 1492, where they were previously not indigenous, and which live in the wild (Geiter, 1999). The correlation of the globalization, climate change and neozoa is striking, when plotting ship traffic, temperature data and registered neozoa of Germany (see Fig. 1).

The principle of species transferred by humans is not new and even goes back to pre-Columbian times. These early introduced species are called 'archaeozoa': The by far earliest archaeozoa is the softshell clam *Mya arenaria*, which was introduced from North America to Europe by the Vikings in 982 (Hessland, 1946; Kinzelbach, 1995). Today, *M. arenaria* is one of the most common bivalves in the North and Baltic Sea (Strasser et al., 1999). The current geological stage of the Baltic Sea is even named after *M. arenaria* (Voß and Dippner, 2017). This is just one example of how an introduced species becomes the dominating species in the new ecosystem. Of course, not all introduced species get established in the new habitat. Only a small fraction reaches high population densities with largely negative impacts on the new ecosystem, which are then called 'invasive species' (Colautti and MacIsaac, 2004; Geburzi and McCarthy, 2018; Sakai et al., 2001).

In Germany, shipping traffic is presently the most important introduction vector for invasive species. 52% of all invasive species in Germany were introduced via

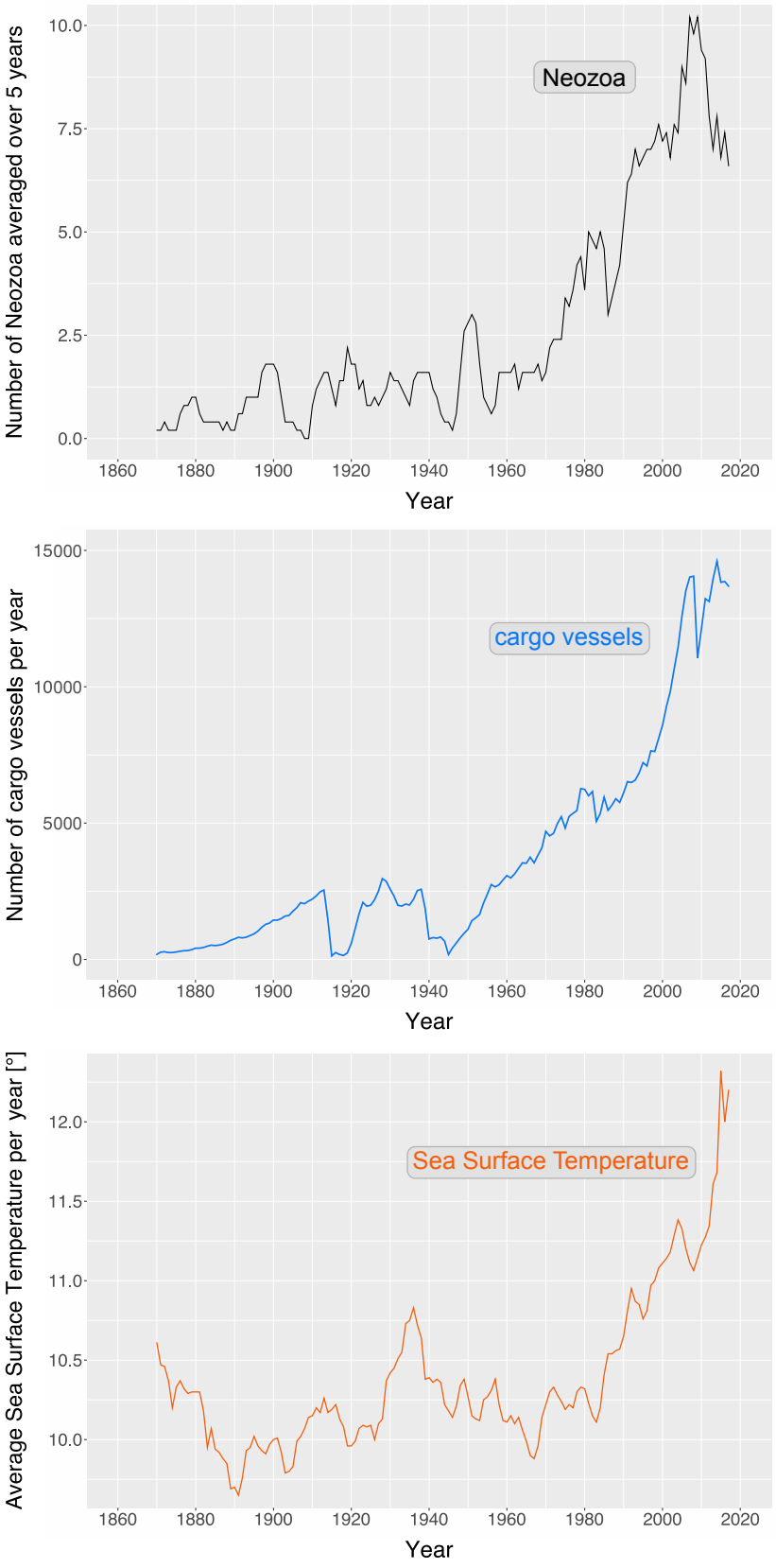

Figure 1: Uppermost graph shows the historical development of the number of neozoa in the North Sea (black); middle graph shows the historical development of the number of cargo ships in the port of Hamburg (blue); lower graph shows the increase of sea surface temperature in the North Sea since 1860

shipping traffic and ballast waters (Nehring, 2005). Investigations have shown that 2.7 million individuals of 4 000 species are released through ballast water into ports of Northern Germany each day (Gollasch, 1996). Gollasch, 2002 also sampled and identified the species on ship hulls and in ballast water in Hamburg and Kiel harbour for four years and found that 68% of the species on the hulls are non-native. Although, only 1.6% of the introduced species become invasive, the impact is still enormous: The round goby *Neogobius melanostomus*, for example, is one of the most wide-ranging invasive fish on earth and originally native to the Black Sea. In 1990, it was first recorded in the Baltic Sea at Puck Bay (Gulf of Gdansk) (Kornis et al., 2012). It is presumed that the larvae were released to the Baltic Sea through ballast waters (Kornis et al., 2012). Since then, it spread rapidly and can now be found in the complete Baltic Sea. Due to its wide salinity tolerance, *N. melanostomus* can inhabit both fresh water, as well as brackish and marine waters (Cross and Rawding, 2009; Gollasch et al., 1999), which is the basic requirement for spreading through the navigable channels across Germany. Today, this species is recorded in the Danube, the Rhine, River Oder and Elbe River (Brandner et al., 2013; Ghomi et al., 2014; Hempel, Thiel et al., 2013; Schomaker, Wolter et al., 2014), being both advantageous and disadvantageous for various species through competition, predation and as prey (Kornis et al., 2012).

Today, invasive species are the major threat to marine biodiversity (Molnar et al., 2008). Nevertheless, the processes and pathways of introduction caused by globalization and the impacts of invasive species on native ecosystems is not yet fully understood. In most cases, invasive species are only noticed, when they are already established in the new habitat and then it is too late to investigate the pathways of their introduction. The same applies for the processes of climate change and its consequences on marine species and ecosystems. The consequences are in some cases visible by coral bleaching and distribution shifts, but the underlying processes and causal relationships are rarely understood.

In these cases, natural history collections are most valuable tools to answer environment-related research questions. Natural history collections store selective specimens that are linked to a specific date and place. Due to several sampling events over time, collections store not only individual specimens, but whole time series of species. Based on these time series, highly relevant scientific questions can be addressed, such as biogeographical changes of marine species in certain environments, either through evolutionary processes in a geological timescale, or by human induced transformations of habitats throughout the last centuries and decades. Often species are collected, which have been known for a long time or are commercially relevant such as *Ostrea edulis* Linnaeus, 1758, the European flat oyster. The species has been a delicacy for centuries, therefore the interest in *O. edulis* and

especially the interest in the well-being of the oyster beds have been high. In the late 19th century, the demand for the European flat oyster increased to such an extent that the German emperor Wilhelm I. at that time asked the former director of the Zoological Museum Kiel (Germany) to find a way to increase the productivity of *O. edulis*. In the course of his research contract, Karl August Möbius travelled across Europe collecting *O. edulis* from different places continuously over several years. His research and collection today represent the basic knowledge about the European flat oyster in German waters (Möbius, 1877).

In addition, Natural history collections often hold time series of species that have caused a commotion, such as the invasive neozoan species *Crepidula fornicata* Linnaeus, 1758 or *Eriocheir sinensis* H. Milne Edwards, 1853. *C. fornicata* or the common limpet slipper was introduced to Europe in the late 19th century and was soon accused to be an oyster pest, since it reproduced massively and competed for food and space with the native oyster *O. edulis* (Yonge, 1960). *E. sinensis* or the Chinese mitten crab, on the other hand, is native to China and came to Europe in the beginning of the 20th century, where it soon spread rapidly and occurred in such high numbers that, combined with their burrowing behaviour, caused massive economic damage to dams and the fishing industry (Panning and Peters, 1933).

By using natural history museum collections, the aim of this doctoral thesis was generally to investigate faunal changes in North and Baltic Sea against the background of climate change and globalization. More precisely, the thesis analyses extinction events, the impact of neozoa on native species and whether long established invasive species experience differentiation or adaptation processes in the new habitat. These research questions were addressed to the locally extinct European flat oyster *Ostrea edulis* and its 'oyster pest' – the common limpet slipper *Crepidula fornicata* – as well as the invasive species *Eriocheir sinensis*, which is successfully established in Germany for over 100 years. With the help of the museum collection material, the distributional changes of these three species have been reconstructed in the North and Baltic Sea since the late 19th century (Fig. 2). For the European flat oyster, the phylogeographical changes were further elucidated with ancient DNA (aDNA) techniques.

This study is part of the MARSAMM project, which is financially supported by the Federal Ministry of Education and Research (BMBF). It is an interdisciplinary project combining thorough collection documentation and innovative analytical methods. In cooperation with the collections of the Senckenberg Research Institute, Frankfurt (Germany) and the collections of further museums along the Northern coast of Germany (NORe e.V.), the marine invertebrate collections of the Zoological Museum Kiel (Germany) was used as the basis of this project. Thus, the MARSAMM project provides a worldwide unique database to document the historical faunal

changes of North and Baltic Sea due to natural and anthropogenic influences. This project aims to reconstruct shifts in species distribution, invasion as well as extinction processes in the light of climate change and globalization.

Figure 2: Images of historic collection specimens of the three species used in this study. **(A)** *Eriocheir sinensis* from the collections of the Zoological Museum Hamburg, ©Jutta Drabek-Hasselmann **(B)** *Crepidula fornicata* from the collection of the Zoological Museum Kiel, ©Fabian Haas **(C)** *Ostrea edulis* from the collections of the Zoological Museum Kiel, ©Jutta Drabek-Hasselmann; scale bars = 2cm

Ostrea edulis

The European flat oyster or *Ostrea edulis* is native to Northern European coasts. Archaeological shells confirm that this mollusc is native to Northern European coasts since the Holstein-Interglacial, a geological period ranging from 260 000 years to more than 440 000 years ago (Barabas et al., 1988; Grahle, 1936). Shells of this age have been found in the collection of the Zoological Museum Kiel during the projects' documentation, which were sampled in the late 19th century in an excavation site in Tarbek, a small town in Schleswig-Holstein (Northern Germany). This excavation site is known to hold whole oyster beds in marine sediments dated back to the Holstein-Interglacial (Grahle, 1936). The latest excursion to Tarbek proves that ancient oyster shells and accompanying fauna can still be found (see Fig. 3).

Figure 3: Images of the sampling excursion to Tarbek (Schleswig-Holstein, Germany) in July 2018. Left picture shows the excavation site on the area of a landfill site (ABE Deponie GmbH). Right picture shows ancient oyster shells and accompanying fauna in marine sediments. ©Jürgen Mnich

In Mesolithic and Neolithic Europe, the oyster seemed to be common in the North, since prehistoric shell middens were found regularly along the coasts of Germany and Denmark (http://zmk.sesam.senckenberg.de/page/index.htm under 'fossil' in 'remarks on object', see Gutiérrez-Zugasti et al., 2011). With the discovery of the shell middens, it was demonstrated for the first time that oysters were used as a food source by humans (Gutiérrez-Zugasti et al., 2011). Later on, the oyster was not only food supply, but was appreciated among the first delicacies by the Romans (Yonge, 1960). The Romans were also the first who cultivated the oyster artificially and traded them within the Empire (Strauch and Thüry, 1985; Thüry, 1990; Yonge, 1960). Humans preference for oysters continued over the centuries, where they were treasured from the North Sea, over the Atlantic coasts from Norway to North East Africa as well as from the Mediterranean Sea to the Black Sea (Gercken and Schmidt, 2014; Yonge, 1960). The increasing demand for oysters in the late 19th century combined with the improving post-industrial technology of fishing had the consequence of depleting and finally largely destroying the beds of the European flat oyster (Yonge, 1960). In the early 20th century, the wild oyster beds of *O. edulis* went extinct in the North Sea – with the exception of the oyster bed in the Limfjord – and they have not recovered since (Gercken and Schmidt, 2014).

With the loss of *O. edulis* in the North Sea, Europeans not only lost a popular delicacy, but the ecosystem is missing a highly important biogenic reef-type organism (Pogoda, 2019). European flat oyster beds represented a characteristic benthic community and had a high number of valuable tasks: they acted as filter-feeders taking up toxic algae and high amounts of nutrients, which led to a better water quality. The structure of the oyster beds increased the bentho-pelagic coupling and species richness providing habitat, food, and protection for numerous invertebrate and fish species (Pogoda, 2019).

European flat oyster beds were mostly situated in sublittoral depths of coastlines or within deeper trenches of the Wadden Sea (Gercken and Schmidt, 2014; Möbius, 1877). Single oyster beds were normally smaller than three kilometres in length, because the expansion was limited by abiotic factors such as salinity, temperature, sediment properties and availability of phytoplankton (Möbius, 1877; Yonge, 1960). But there was also an exception: at depths of up to 80 metres between the German island Helgoland and the Dogger Bank, a huge oyster bed existed that was 100 to 1000 times as large as the shallow oyster beds (Berghahn and Ruth, 2005; Gercken and Schmidt, 2014). In these depths, abiotic factors such as salinity and temperature are stable.

The temperature is primarily responsible for the more northern distribution of the genus *Ostrea*, since it has a great influence on spawning and species belonging to this genus are able to reproduce in colder waters than other oysters (Yonge, 1960). The European flat oyster is a protandric hermaphrodite, which means that individuals change their sexes from an initial male phase to females and back, alternating throughout life (Möbius, 1877; Yonge, 1960). The process of sex change and the fecundity of *O. edulis* has been studied intensively (H. Cole, 1942; Orton, 1933), so the process is described only briefly here. As soon as the temperature reaches 15°C, both sexes spawn. Males release sperm packages into the water, where the spermatophores are spread over the oyster bed and are taken up by females through the inhalation siphon (Gercken and Schmidt, 2014; Yonge, 1960). Females pass the spermatophores into the mantle cavity, where the mature eggs are fertilized. In contrast to other oysters, the genus *Ostrea* is ovoviviparous. The oysters do not release their fertilized eggs into the water, but transport them into the inhalant chamber of the female, where the eggs remain another eight to 15 days depending on the temperature, until the larvae are liberated (Gercken and Schmidt, 2014; Korringa, 1952; Yonge, 1960). The veliger larvae liberated are planktonic for seven to twelve days and their distribution depends on the currents. During this planktonic stage, they search for a suitable place to settle down (Korringa, 1952; Yonge, 1960).

Despite the high amount of veliger larvae released every reproductive cycle, only a small number of larvae survive to become spat (Yonge, 1960). After settlement, young oysters are also exposed to greatest dangers such as predators, competitors, parasites and the effects of physical factors (Yonge, 1960). Yet adult oysters face threats as well. Strong winters like the ones in 1728-29, 1829-30 and 1939-40 destroyed whole oyster beds in the Wadden Sea and Thames Estuary as temperatures fell to the freezing point and the oysters were exposed to ice during low tide (Hagmeier and Kändler, 1927; Möbius, 1877; Yonge, 1960). But the greatest threats to adult oysters are overfishing, introduced competitors and diseases. Due to the increased demands for the delicacy, the fishing returned millions of oysters since

the middle of the 19th century until fishing was no longer profitable (Möbius, 1877; Schümer, 1990; Yonge, 1960). In order to maintain the fishing industry, the Pacific oyster *Magallana gigas* (Thunberg, 1793) was introduced to Europe in 1870, where it was believed to not reproduce, because the necessary higher spawning temperature would not be reached in European waters. However, the Pacific oyster soon spread along the coasts and is today the most common oyster. Along with *M. gigas*, another invasive species was introduced. The common limpet slipper or *Crepidula fornicata* is a filter feeder just like *O. edulis* and has long been suspected to be a competitor of the native oyster, but a displacement effect could not be proven (de Montaudouin et al., 1999; Thouzeau et al., 2000). However, a new parasite was introduced to Europe in the late 1970s that is still causing major problems to oyster beds and farming: The haplosporidian parasite *Bonamia ostreae* Pichot, Comps, Tigé, Grizel & Rabouin, 1980, that induces bonamiasis disease, spread across Europe annihilating entire oyster beds (McArdle et al., 1991). Because this disease spreads rapidly between individuals, it is particularly destructive within oyster farms that keep oysters in high density (Kennedy and Roberts, 1999). Therefore, oyster farms have suffered severe losses and experienced mortality levels of over 80% on the Atlantic coast of France (Figueras, 1991).

Although many threats to the native oyster are known today, scientists are still speculating why the oyster became extinct in the North Sea and why it has not returned until today. To consult the collections is going to answer some of those questions.

Crepidula fornicata

The common limpet slipper or *C. fornicata* is a marine benthic gastropod that can grow up to six centimetres (Blanchard, 1997). The species got its vernacular name because of the shape of its shell, which looks like a slipper when seen from below (see Fig. 2B) (D. Thieltges et al., 2003). The peculiar shape of their shell also allows them to attach themselves tightly to the shell of another individual of *C. fornicata*. This way, the animals form a tower of up to 14 animals, whereby the arrangement of the individuals has a predefined order: the lowest and largest individual is female and the uppermost smaller individuals are males (D. Thieltges et al., 2003). Moreover, *C. fornicata* is a protandric hermaphrodite just like *O. edulis*, which can be observed in the tower of individuals (Coe, 1936; Hoagland and KE, 1978; Wright, 1988): the individuals between the lowest female and the upper males are in the middle of changing sex (D. Thieltges et al., 2003). In contrast to *O. edulis*, the snails change their sex only once in life.

Another characteristic that *C. fornicata* shares with *O. edulis* is the filtering feeding

method (B. Werner, 1953). But unlike *O. edulis*, *C. fornicata* uses a complex and highly efficient feeding mechanism. By slightly raising the shell water enters and the incoming plankton is captured on the gill filaments. The animal produces plenty mucous threads that entangle the plankton particles, which are then reeled into cords and transported anteriorly to the head. As soon as the mucous threat reaches the head, the radula grabs the cord and transports it into the mouth. This way of plankton capture is highly efficient; however, the ingestion is less effective. The caption of the food cord by the radula is often missed or rejected, sending this mucous plankton material to the benthic surface leading to an enrichment of soft sediments on the ground (Shumway et al., 2014). Nevertheless, this feeding method allows the common limpet slipper to find enough food to develop large populations, unlike other grazing patellids (Hoagland and KE, 1977). Because *C. fornicata* co-occurs with the European flat oyster in the same habitat and shares its feeding mode, the common limpet slipper was perceived as a competitor to *O. edulis* (Ankel, 1935; Blanchard, 1997; Korringa, 1951; Linke, 1947; Orton, 1927; Schuster, 1951; B. Werner, 1948). Additionally, former studies documented that *C. fornicata* is not only a competitor but also a predator to *O. edulis*, since adult snails not only feed on phytoplankton, but also on zooplankton. They can ingest larvae of at least 800 µm in shell length, which includes the veliger larvae of *O. edulis* (Korringa, 1951; Pechenik et al., 2004).

C. fornicata is a new competitor to the European flat oyster, since it is originally native to the east coast of North America from Prince Edward Island (Canada) to Texas and the Bahamas (Collin, 2001; Fretter and Graham, 1981; Hoagland and KE, 1979). It was first introduced to Europe as a stowaway together with *M. gigas*, the Pacific oyster, in the 1870s (Blanchard, 1997; Chipperfield, 1951; McMillan, 1939; Orton, 1950; D. Thieltges et al., 2003; D. W. Thieltges et al., 2004). Literature documented the new species in Liverpool Bay in 1872 for the first time (McMillan, 1939) and it soon spread along the coast of Great Britain (H. A. Cole, 1952; W. Cole, 1915; Crouch, 1893; Walne, 1956; Yonge, 1960). *C. fornicata* was probably introduced multiple times or was transported from Great Britain to the European continent, as it was observed in Belgium in 1911 (Adam and Leloup, 1934). From there, it spread rapidly southwards to France and Spain (Blanchard, 1995; Rolán et al., 1985; Rolán et al., 1983), and northwards over the Netherlands, Germany, Denmark and Norway to Sweden (Adam and Leloup, 1934; Hessland, 1951; Spärck, 1951; B. Werner, 1948). But not only the speed of the spread and establishment of *C. fornicata* in Europe was alarming, but also the mass of occurring individuals on the coasts. Alone on the coast of Essex (England), up to 446 individuals per square metre were recorded, covering the oyster beds completely (Yonge, 1960; see Fig. 4). These exploding numbers soon after introduction gave them the byname 'oyster pest'. *C. fornicata* is now a very successful invasive species. This species found favourable environmental

conditions in Europe from Scandinavian coasts to the mid Mediterranean and with global warming, its geographic range is expected to continue to expand with rising seawater temperatures (Beninger et al., 2007; Blanchard, 1997).

Figure 4: Historic photography of clearing individuals of *C. fornicata* from derelict English oyster beds, showing immense numbers of the introduced species. Image taken from Yonge, 1960.

Due to the ecological and economic impact of this species to the new environment, *C. fornicata* has been the centre of interest of oyster farmers especially since the beginning of its introduction. On the basis of the museum collections, the historical distribution and spread of the invasive species can be reconstructed. Thus, the possible influence of *C. fornicata* on the extinction of the European flat oyster in the North Sea can also be demonstrated.

Eriocheir sinensis

The Chinese mitten crab *Eriocheir sinenis* is native to Taiwan and along the east coast of China from the Korean peninsula in the south to Vladivostok (Russia) in the north. Its distribution range partly overlaps with that of two other species, *E. hepuensis* and *E. japonica*. All three species are delicacies and are of great commercial interest especially in China (Naser et al., 2012; Sun et al., 2005; C. Wang et al., 2008; Xu et al., 2009). These eubrachyuran crabs belong to the superfamily Grapsoidea and are grouped within the Varunidae (Kitaura et al., 2002; Martin and Davis, 2001;

Schubart et al., 2000). Grapsoidea occur worldwide and are adapted to a wide variety of semi-terrestrial, freshwater, and marine habitats.

In comparison to the molluscs *O. edulis* and *C. fornicata*, the Chinese mitten crab is not a purely marine organism, but a catadromous species that spends most of its life in coastal rice fields or riverine habitats in inland areas (Panning, 1939). There, it commonly inhabits self-dug tunnels along the banks of rivers and lakes, where it seeks shelter during the day. These tunnels can be found anywhere between the flood mark, under reed banks to below the low-water mark. Up to 30 tunnels per square metre are common (Dutton and Conroy, 1998; Peters, 1933a, 1938). Chinese mitten crabs are most active during the dark (Fu et al., 2017; Gilbey et al., 2008). They are omnivorous and opportunistic and feed on animals as well as microphytes and macrophytes (Jin et al., 2003; Jin et al., 2001; Mao et al., 2016).

Because of its omnivorous diet and aggressive interspecies behaviour (Jin et al., 2003; Jin et al., 2001; Mao et al., 2016; Rudnick et al., 2000), *E. sinensis* is a very successful invasive species (Lowe et al., 2000). In 1912, this species has been accidentally introduced to Northern Germany probably via the ships' ballast waters (Cohen and Carlton, 1997; Peters, 1933b). The first individual was discovered in a tributary of the River Weser in Northern Germany, the second in the River Elbe two years later. Soon after, self-sustaining populations of the Chinese mitten crab were established in both river systems (Marquard, 1926; Panning and Peters, 1933). During the 1930s, the crabs occurred in such massive numbers that 1935 alone, more than 12 000kg of this crab was caught at a dam on the Weser (Panning, 1939). After a dramatic reduction in abundance during the 1940s, which is still unexplained, the mitten crab population showed cyclic increases again in the 1950s, early 1970s, early 1980s and have been increasing again since 1993 (Gollasch, 1999). This species spread also further north and is now abundant at the coast of the Baltic Sea as well. Today, *E. sinensis* is an established species in Northern Germany and is also found throughout most of western, central and northern Europe (Rudnick et al., 2000). The wide spread in the new range can be explained by the absence of serious enemies and competitors as well as the rising water quality in several waters (Fladung, 2000).

In both the native and introduced range, the Chinese mitten crab spends one to five years in fresh water, before they become sexually mature (Hymanson, 1999; Jin et al., 2002; Veldhuizen and Stanish, 1999; Zhao, 1999). After puberty molt, they start to migrate downstream in late summer until winter in order to reproduce (Cheng et al., 2008; Rudnick et al., 2003; Sui et al., 2009; T. Zhang et al., 2001). During the reproductive migration, the gonads of the crabs gradually develop (Bentley, 2011; L. Zhang et al., 1973; T. Zhang et al., 2001). The males usually arrive first on marine waters and the females follow within a month of the males (Anger, 1991). At their destination, they become mature, copulate and subsequently spawn (Bentley, 2011;

L. Zhang et al., 1973; T. Zhang et al., 2001). Whereas the males die after copulation, the females carry from 250 000 to 1 000 000 eggs attached on the pleopods (Panning, 1939; Zhao, 1999). But as soon as the larvae hatch in spring, the females die as well (Zhao, 1999). The Chinese mitten crab larvae are planktonic for about 1 – 2 months and undergo one prezoea and five zoea larvae stages in marine waters. After the metamorphosis to the megalopa stage, they settle down on the bottom of marine coastal areas. Here, the megalopa gets transported by currents toward the mouths of the river estuaries, where it develops into a juvenile crab (Kinne, 1971). Juveniles start to migrate upstream and can undertake enormous journeys. They have been found as far as 1 250 km from the coast in freshwater streams (Dan et al., 1984). Young juvenile Chinese mitten crabs are mainly found in tidal brackish and fresh water areas, older juveniles are located further upstream. This successful dispersal in freshwater as well as brackish water is facilitated through its holo-euryhalinity (Kinne, 1971).

At the Baltic Sea, *E. sinensis* shows a different reproductive behaviour and physiology compared to the native range. Despite the low salinity, the Chinese mitten crabs are able to successfully reproduce in the brackish Baltic Sea. In contrast to larvae of other populations, the Baltic Sea larvae reveal a unique adaptation and produce an additional sixth zoea larval stage under these unfavourable salinity conditions (Anger, 1991; Montú et al., 1996). Otto, 2012 was able to find both egg-carrying females as well as zoea larvae stage II and juvenile crabs in adjacent rivers in the Kiel fjord (Germany). Additionally, post-spawning females migrate back into the rivers and do not die after the larvae hatch (Anger, 1991). This proves that *E. sinensis* has successfully adapted to the altered conditions and is able to reproduce in the Baltic Sea (Otto, 2012).

Because of these differences in reproductive behaviour and physiology between the brackish and marine water populations, it is of great scientific interest to reconstruct whether these differences are based on multiple introductions to Europe or rapid adaptations within the new range. Because the introduction process of *E. sinensis* in Europe is well documented in museum collections, this species is a likely candidate for this study.

Aims of the thesis

With the help of the generated database of the cooperating museums, the public genetic databases (NCBI GenBank, BOLD) and the methods of aDNA, the following questions were addressed in my doctoral thesis:

- **Chapter 1**:

  Reconstruction of the historical distribution of both *Ostrea edulis* and *Crepidula fornicata* based on museum collection material: Had *Crepidula fornicata* a negative impact on the survival of *Ostrea edulis* in the North Sea?

- **Chapter 2**:

  Reconstruction of the historic phylogeography of *Ostrea edulis* in Europe based on aDNA mitochondrial genomes: Can the historical population structure of *Ostrea edulis* explain the extinction of the native oyster in the North Sea today?

- **Chapter 3**:

  Re-evaluation of the genetic diversity of *Eriocheir sinensis* throughout its native and invaded range based on data of public genetic databases: Can the source population of *Eriocheir sinensis* in its native range be identified? Will the genetic investigation be able to determine whether multiple introductions or rapid adaptations are responsible for the biological differences of crabs in Northern Europe?

Chapter 1

Coming and going – Historical distributions of the European oyster *Ostrea edulis* Linnaeus, 1758 and the introduced slipper limpet *Crepidula fornicata* Linnaeus, 1758 in the North Sea

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Abstract

Natural history collections are fundamental for biodiversity research as well as for any applied environment-related research. These collections can be seen as archives of earth's life providing the basis to address highly relevant scientific questions such as how biodiversity changes in certain environments, either through evolutionary processes in a geological timescale, or by man-made transformation of habitats throughout the last decades and/or centuries. A prominent example is the decline of the European flat oyster *Ostrea edulis* Linneaus, 1758 in the North Sea and the concomitant invasion of the common limpet slipper *Crepidula fornicata*, which has been implicated to have negative effects on *O. edulis*. We used collections to analyse population changes in both species in the North Sea. In order to reconstruct the change in distribution and diversity over the past 200 years, we combined the temporal and spatial information recorded with the collected specimens contained in several European natural history collections. Our data recover the decline of *O. edulis* in the North Sea from the 19th century to the present and the process of invasion of *C. fornicata*. Importantly, the decline of *O. edulis* was nearly completed before *C. fornicata* appeared in the North Sea, suggesting that the latter had nothing to do with the local extinction of *O. edulis* in the North Sea.

Introduction

Ostrea edulis Linnaeus, 1758 or the European flat oyster has been used as food source by humans for centuries. Shells have been found during excavations of Mesolithic kitchen mid- dens and in settlement remains of Vikings as well as Romans (Gercken and Schmidt, 2014; Lüttschwager, 1954; Neudecker, 1990; Thüry, 1990; Yonge, 1960). In the German North Sea, oyster fishery was one of the first recorded commercial fisheries in the 12th and 13th century (Gercken and Schmidt, 2014; Lotze, 2007). In later centuries, the demand for oysters remained high and oyster fishery along the North Sea coasts continued to increase and become more efficient (Gercken and Schmidt, 2014). However, due to the constant fishing of adult oysters, the

spat fall declined over the years (Gercken and Schmidt, 2014). In 1868, the Prussian King and German Emperor Wilhelm I. decided to make the attempt to breed oysters commercially. Karl August Möbius, professor of Zoology and director of the Zoological museum in Kiel (Germany) at the time, received a research contract to investigate the possibility of artificial oyster farming in the German North Sea. Additionally, he was asked to find a way to improve the productivity of the native oyster banks due to the increased demand for oysters. Therefore, he visited oyster beds in the North Sea on the coasts of Germany, France and England to fulfil his contract and brought many specimens of *O. edulis* back to Kiel and other museums. He concluded that oyster farming as it was carried out in France and Great Britain was not possible at the German coast, and oyster production in the North Sea was already maximized (Möbius, 1877). In 1882, oyster fishery had to be stopped on the German island Sylt, because the oyster beds were overaged (Schümer, 1990). Despite measures to protect the oyster population from overexploitation, oyster fishery continued to decline dramatically. Most natural populations of the European oyster went extinct in the North Sea in the 1940s (Gercken and Schmidt, 2014; D. Thieltges, 2003).

Until the beginning of the 20$^{\text{th}}$ century, *O. edulis* was commonly found in the shallow regions of the Atlantic coasts from Norway to North East Africa as well as from the Mediterranean Sea to the Black Sea (Lapegue et al., 2006; Ranson, 1948; Strauch and Thüry, 1985; Yonge, 1960). Nowadays, *O. edulis* is found in large numbers in the Limfjord, which is the only surviving natural population in the North Sea, but mostly in commercial oyster farms on the European Atlantic coast or in very small and endangered natural populations in, for example, Norway and Sweden (Gercken and Schmidt, 2014). Recently, there are observations of *O. edulis* in Danish offshore wind farms, hinting at a possible re-colonization of the North Sea (Drinkwaard, 1998; Gercken and Schmidt, 2014; Korringa, 1952; Yonge, 1960, 1964).

It remains a mystery why the European flat oyster has not returned to the North Sea sooner, since the environmental conditions for a successful establishment have not changed. *O. edulis* generally inhabits the intertidal zone to a depth of 20 metres, but has been found at depths up to 50 metres (Gercken and Schmidt, 2014; Lapegue et al., 2006). The individuals often occurred in large beds on muddy-sand, muddy-gravel and firm grounds, where they feed on plankton (Lapegue et al., 2006; Möbius, 1877). *O. edulis* normally needs salinities above 30‰, but for short periods of time, it tolerates salinities between 16‰ and 19‰ in estuaries (Gercken and Schmidt, 2014; Hutchinson and Hawkins, 1992). The European flat oyster is a protandric hermaphrodite, generally changing sex once a year in the North Sea (Gercken and Schmidt, 2014; Lapegue et al., 2006). An *O. edulis* individual may begin the new season either as a male or as a female (Yonge, 1960). Some *O. edulis* function as males early in

the spawning season and change later to females before becoming males again in the next season. Female individuals produce up to 1 million eggs per spawning and release them into the inhalant chamber, where they are fertilized by the indrawn sperm of neighbouring male individuals (Yonge, 1960). Following an incubation period of 8 – 10 days, depending on water temperature, larvae are released into the environment and spend 8 to 10 days as pelagic dispersal stages before they settle down on a suitable substrate. Appropriate larval growth and survival rates are obtained in 20‰ salinity and a minimum temperature 15°C – 16°C, although they can survive at salinities as low as 15‰(Gercken and Schmidt, 2014; Lapegue et al., 2006).

Numerous reasons for the extinction of natural oyster beds are hypothesized. The overexploitation is the most favoured reason, but strong winters, diseases and invasive species as competitors have been speculated to play a role as well (Drinkwaard, 1998; Gercken and Schmidt, 2014; Hagmeier and Kändler, 1927; Möbius, 1877; D. Thieltges, 2003). One invasive species that was thought to threat the oyster populations was *Crepidula fornicata* Linnaeus, 1758, the common limpet slipper (D. Thieltges, 2003). This snail is commonly found in the intertidal zones and is also a filter feeder, hence was feared to be a feeding competitor for *O. edulis* (Ankel, 1935; Blanchard, 1997; Korringa, 1951; Linke, 1947; Nehring and Leuchs, 2000; Orton, 1927; Schuster, 1951; D. Thieltges, 2003; B. Werner, 1948). It was introduced to Europe together with the Pacific oyster, *Magallana gigas* (Thunberg, 1793), that was imported for the first time in 1870 to revive the European oyster fishery (Blanchard, 1997; D. Thieltges, 2003). *C. fornicata* was first discovered on the German coast in 1934 (Ankel, 1935, 1936; Blanchard, 1997; D. Thieltges, 2003). While it was also introduced to other regions of the world, it spread rapidly in Europe, where it is established now and occurs from South Norway to Spain (D. Thieltges, 2003).

To reconstruct the demographic distribution and abundance of both *O. edulis* and *C. fornicata* in the past, we surveyed museum collections across Europe. The historical collections of museums are the basis of taxonomic and biogeographic research as well as applied environmental research (McCarthy, 1998; Shaffer et al., 1998; Suarez and Tsutsui, 2004). They are a valuable heritage in their historical, biological and cultural references. The collections document the dynamics of change of the biosphere. They preserve evidence of changes in biodiversity, either through evolutionary processes in geologically long or short periods, or through man-made transformation of habitats (Suarez and Tsutsui, 2004). Thus, the collections have, among other things, a function as ecological archives by documenting ecological condition in a particular place and time.

The aim of our study is to investigate a possible connection between the extinction of *O. edulis* in the North Sea and the arrival of the invasive common limpet slipper *C. fornicata*. To this end, we reconstruct the historical distribution of *O. edulis*

Table 1: Numbers of collected specimens and collection records of *Ostrea edulis* and *Crepidula fornicata* from cooperating museums and from public databases of the museums in London (GB), Leiden (Netherlands) and Paris (France).

Museum/collections	**Museum acronym**	**Records of *O. edulis***	**Indiv. of *O. edulis***	**Records of *C. fornicata***	**Indiv. of *C. fornicata***
Senckenberg Natural History Collection, Dresden, Germany	SNSD	1	1	8	20
Senckenberg Natural History Museum, Frankfurt, Germany	SMF	2	175	5	8
Zoological Museum Greifswald, Germany	ZIMG	2	> 3	/	/
Centre of Natural History, Hamburg, Germany	ZMH	20	68	/	/
Zoological Museum, Kiel, Germany	ZMK	93	509	11	107
Naturalis Biodiversity Center, Leiden, Netherlands	NMNL	146	851	97	> 495
Natural History Museum, London, UK	NHML	3	6	10	70
Museum for Nature and Environment, Lübeck, Germany	MNUL	3	18	1	5
Zoological Collections of the University Rostock, Germany	ZSRO	14	> 85	/	/
German Oceanographic Museum, Stralsund, Germany	DMM	6	28	13	> 34
Muséum National d'Histoire Naturelle, Paris, France	MNHN	5	5	/	/

in the North Sea from the 19th century to the present based on specimens of museum collections.

Material & Methods

Data preparation

We used approximately 1 750 individual clamshells of *Ostrea edulis* and 739 individual shells of *Crepidula fornicata* collected between the 1820s and 2018. Table 1 lists the number of collected specimens from every museum used in this study, where they are permanently reposited. The details of the location of every specimen are given in the Tables S1 – S5 (supplementary material). Records for both species were reviewed and the identification was verified. In order to reconstruct all relevant specimen data, we further surveyed the archives of the respective museums. In order to determine whether *O. edulis* was collected alive or dead, the shells were consulted unless this information was given on the original labels. If the right and left valve of the shell or musculature tissue were present or the periostracum was intact, the shells were labelled as being found alive. On the contrary, if shells were single, overgrown on the inner surface of the shell by epifauna, damaged or heavily infested by

parasites the shells were labelled as being found dead. These findings are referred to as 'empty shells' later on. If neither of these circumstances occurred or the material could not be checked, the status was considered to be unknown.

To determine species distribution, the original geographic coordinates were used when provided. When no geographic coordinates were given, google maps was used to infer the geographical coordinates from the location description. In some cases, the descriptions of localities were too ambiguous, hence two values for each longitude and latitude were calculated: The most possible northern and southern for latitude and the eastern and western most possible location for longitude.

Plotting the data on maps

To reconstruct the historical distribution of the species in the North Sea, only the most northern and western coordinates for each specimen were used. The maps were constructed in R Studio version 1.1.453 (R. Team, 2016) and the additional package 'ggplot2' was used for graphical output (Wickham, 2009). The graphics were edited using Affinity Designer (version 1.6.1, Serif, Nottingham, England).

Graphical output and statistics

All analyses were conducted in R Studio version 1.1.453 (R. Team, 2016) using the 'ggplot2' package for graphical output (Wickham, 2009). To investigate the relationship between the collection records of live *O. edulis* and *C. fornicata* over time, we calculated the number of records per decade and plotted the results on a linear plot using the 'ggplot2' package. To analyse if the annual number of shells collected declined over time, we modelled a linear regression with the 'lm' function of the 'stats' package between the collection years and the number of shells collected. Therefore, we had to exclude the collection records without information on numbers of shells. We also calculated the relationship between the collection year and whether *O. edulis* was found dead or alive using a logistic regression. We used the 'glm' function of the 'stats' package to model a logistic function. In order to minimize bias, we calculated both models with the whole dataset and without the oysters collected by Möbius, since he collected consistently over 17 years.

Results

The investigated museum collections contain specimens of *O. edulis* from 19th to the 21st and *C. fornicata* from the 20th to 21st century with a wide distributional range across Europe (Table 1; see Fig. S1). In the following, we will focus on the historical

distribution of *O. edulis* found alive and *C. fornicata* in the North Sea in the 19th, 20th and 21st century (see Figs. 5 – 7).

The earliest collected specimen of *O. edulis* available dates back to the 1820s and was known to be six years old when collected in the Northfrisian Wadden Sea (Fig. 5A). Between the years 1868 and 1885 the full North Sea area was sampled and *O. edulis* was collected alive from the coasts of Denmark, Germany, the Netherlands, France and England (Fig. 5B). For instance, the 'true native' market oyster was bred in the estuaries of the river Roach (Essex, England) and river Colne, a tributary of the Thames. Another market oyster called 'the Nore' that had already gone extinct at that time, was collected from the Thames estuary in 1869. An oyster labelled to be very old was collected from the Herne Bay (Kent, England), although it originated from the English Channel and was put there to refresh the oyster beds in Herne Bay (Fig. 5B).

Between 1868 and 1886, *O. edulis* was commonly found in the German 'North Frisian Wadden Sea' and at the West Coast of Schleswig-Holstein (Germany), where several native oyster beds were known and documented (Gercken and Schmidt, 2014; Hagmeier and Kändler, 1927) (see Fig. 5B). Some of those individuals were collected from the age of 14 days after metamorphosis to 30 years in short intervals. As an example, oysters of the age of one to two years were collected on stones on the oyster bed 'Morsum Odde' after a very mild winter. There, they were flooded 2.5 metres at high tide and lied on dry ground on low tide. Others were collected with parasites, for example, 15 individuals were infested by boring sponges and had barnacles attached to their shells when they were collected at the oyster beds of Sylt in August 1876. *O. edulis* was sampled in the Oosterschelde labelled as market oysters in March 1878 (Fig. 5B).

In 1903 and 1905, *O. edulis* were sampled in the North Sea by the 'commission of the scientific investigation of the German seas' on behalf of the former German royal ministry of agriculture (Fig. 5C). Those oysters were dredged alive in depths of about 30 to 78 metres by the research vessel 'Poseidon'. After 1905 the amount of live oysters decreased and empty shells were found at the coasts of the Netherlands, Germany, Lebanon, Denmark, Italy, France, Croatia and Greece (Fig. 5 – 7). Few live oysters were collected in the North Sea part of 'Oestergronden' in 1939 (Fig. 6A). Cultivated individuals of *O. edulis* were bought from the oyster farm in Galway (Ireland) in 2017 (see Fig. S1).

The results show that the number of museum records of live oysters had already declined dramatically when the records of *C. fornicata* increased (Fig. 8A). According to the results of the logistic regression model, the number of collection records of *O. edulis* per year declined significantly over time (p-value < 0.05, Table 2, see Fig. 8B). The number of museum records per decade of *C. fornicata* in the North Sea

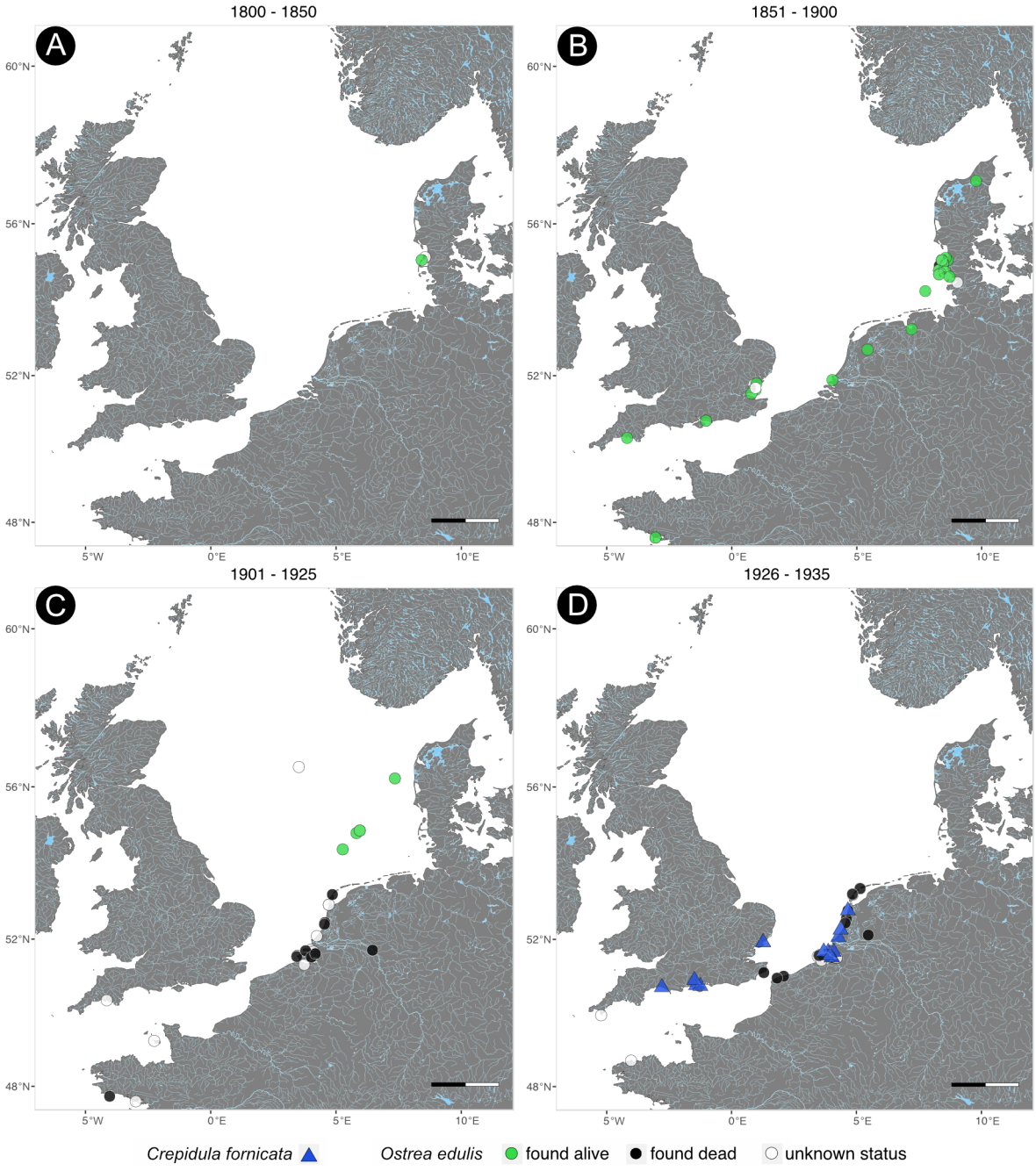

Figure 5: Historical distribution of *Ostrea edulis* and *Crepidula fornicata* from the 1820s to 1935. Time series of the distribution of both species in the North Sea. *O. edulis* was mapped according to its sampling status. Scale bar = 100km.

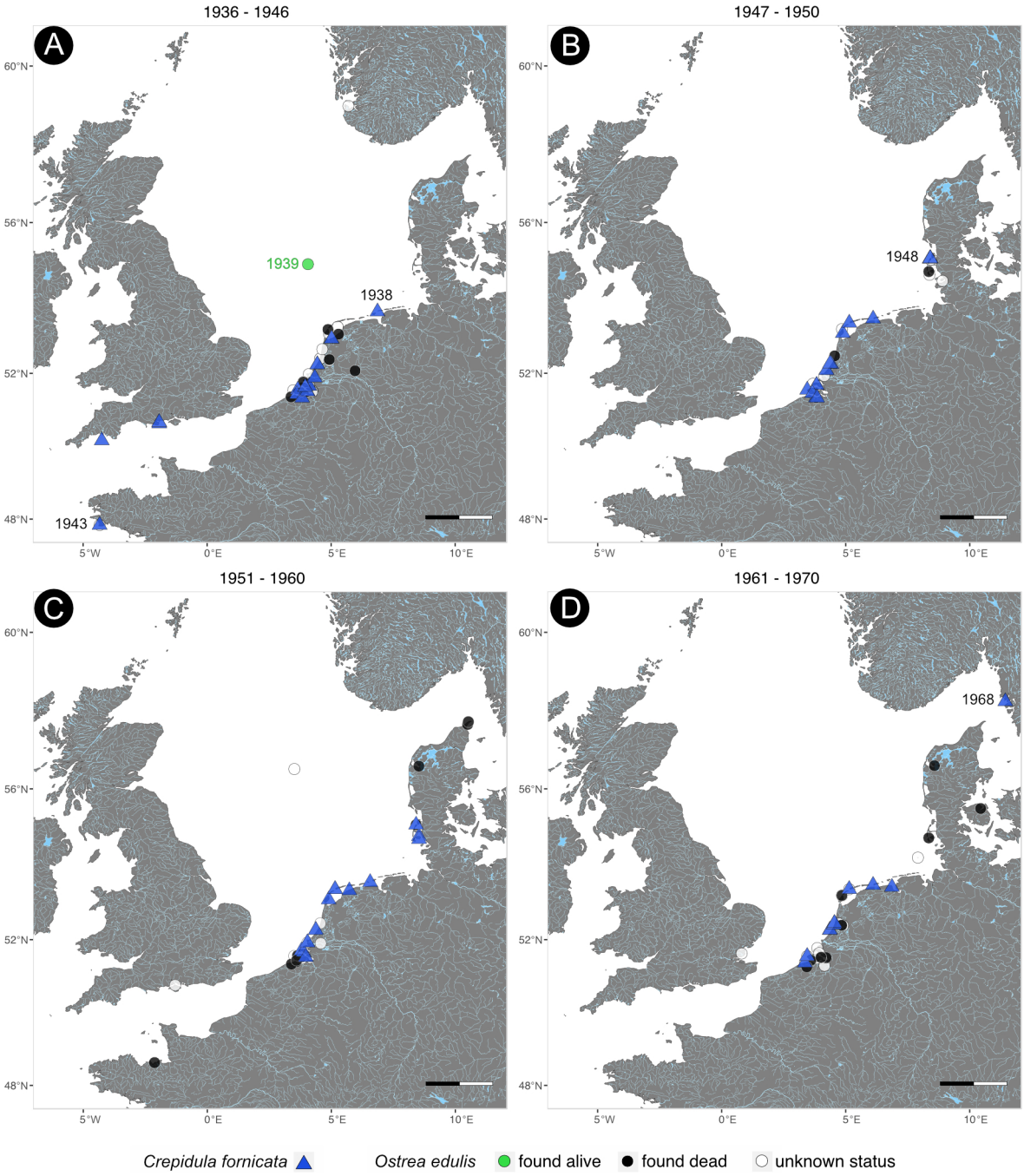

Figure 6: Historical distribution of *Ostrea edulis* and *Crepidula fornicata* from 1936 to 1970. Time series of the distribution of both species in the North Sea. *O. edulis* was mapped according to its sampling status. Scale bar = 100km.

Figure 7: Historical distribution of *Ostrea edulis* and *Crepidula fornicata* from the 1971 to 2018. Time series of the distribution of both species in the North Sea. *O. edulis* was mapped according to its sampling status. Scale bar = 100km.

Table 2: Detailed results of the logistic regressions from the dataset of *Ostrea edulis*.

	Estimated coefficient	Standard error	t-value	p-value
complete data base				
intercept	−191.91907	35.65546	−5.383	7.34e-08 ***
year	0.10088	0.01874	5.384	7.28e-08 ***
Without Möbius oysters (1868 - 1885)				
intercept	−320.74965	99.86855	−3.212	0.00132 **
year	0.16788	0.05212	3.221	0.00128 **

Regressions were calculated with the complete dataset and without the oysters collected by Möbius. Provided are estimated coefficients, standard errors, t-values and p-values for collection years as a function of the number of shells collected. Note: high significance = ***; firm significance = **

is represented in the logistic regression plot by the size of *C. fornicata* shells (Fig. 8B). These results remain significant when the collections of Möbius were removed from the dataset (see Table 2). However, the linear regression model reveals that the number of individual shells of *O. edulis* collected alive did not decrease over the years (p-value > 0.05, see Fig. S2, Table S6). This result is comprehendible as the number of individual oyster shells added to a museum collection were sampled in random numbers since they were collected by different collectors but a collection record of an oyster shell represents always a finding of the animal at a place.

C. fornicata was first recorded at Camperduin-Petten (the Netherlands) on 5th September 1926 and in Davercourt (Essex, England) on 14th September 1929 (Fig. 5D). From there, it spread along the coasts of England and the Netherlands until *C. fornicata* was found at the French Atlantic coast and at the North Sea border to Germany in the late 1930s (Figs. 5D and 6A). In August 1948, it was collected at Königshafen on the German island Sylt providing evidence for the first individual on Sylt (Fig. 6B). *C. fornicata* spread again further north and was first documented at the Gullmarnfjord (Sweden) on 11th October 1968 with a salinity of 32 (Fig. 6D). On 10th September 1990, the slipper limpet made its way into the Kattegat and was found at the beach of Lyngså (Denmark, see Fig. 7B). Today it can be found along all coasts of the North Sea from France to Sweden (Fig. 7C).

Discussion

Our study shows a significant decrease of live individuals of *O. edulis*, a significant increase of empty oyster shells as well as individuals of *C. fornicata* in the North Sea at the beginning of the 20th century (Figs. 8A and 8B). Importantly, we can show that *C. fornicata* appeared after the breakdown of the *O. edulis* population in the

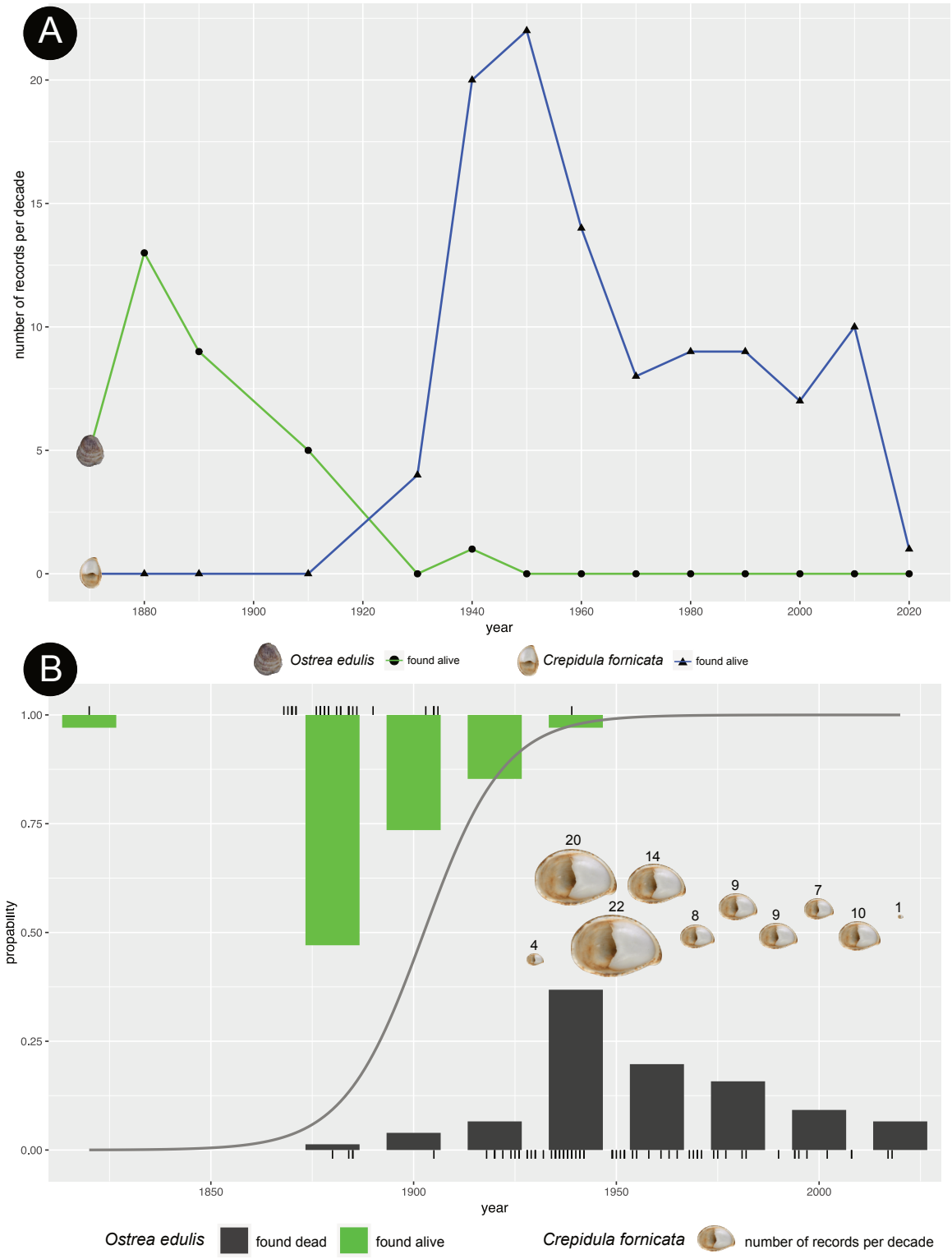

Figure 8: Graphical output of *Ostrea edulis* and *Crepidula fornicata*. **(A)** Line plot of the collection records per decade of live *O. edulis* and *C. fornicata* from the North Sea housed in natural history collections over time. The values on the y-axis display the number of records collected each decade. The values on the x-axis display the year of sampling. **(B)** Logistic regression plot of the collection records of *O. edulis* in the North Sea over time. Additionally, the number of museum records of *C. fornicata* per decade is displayed. The values on the y-axis display the conditional density of oysters found alive (= 1) or dead (= 0). The values on the x-axis display the year of sampling. A logistic regression line shows the decreasing probability of oysters found alive over the years.

North Sea. The number of museum specimens of *C. fornicata* increased in the 1920s (Fig. 8A). At that time, the number of collection oysters had already declined dramatically (Fig. 8A).

At the beginning of the 19th century, few *O. edulis* specimens were collected by museums (Figs. 5A and 8B). This contradicts the literature stating that the European flat oyster was commonly found in the North Sea (Gercken and Schmidt, 2014; Möbius, 1877). For example, oysters were fished with estimated annual catch rates of over 20 million in Great Britain at the end of the 18th century (Fullarton, 1891; Yonge, 1960). One explanation for this discrepancy between literature and our results could be the fact that few *O. edulis* specimens were collected and catalogizied in museum collections in the early 19th century. Native and common species are often underrepresented in museum collections because of selective sampling (Guralnick and Van Cleve, 2005). In most cases only rare and outstanding species were collected to document the change in ecosystems (Guralnick and Van Cleve, 2005). Since a change in the abundance of *O. edulis* was not expected due to the belief in inexhaustable oyster beds, this species was uninteresting for museum collections. In addition, older collections and their documentation are often lost. This is an inherent bias of museum collections that cannot be avoided, only recognized.

By the middle of the 19th century, an abundance of live *O. edulis* was collected, but no empty shells are recorded (Figs. 5B). These records trace back to Karl August Möbius, professor of Zoology and director of the Zoological museum in Kiel (Germany) at the time. He only collected live oysters, because he was interested in the oysters' habitat requirements. This is also an example of biased sampling, because K. A. Möbius was collecting with a clearly defined question of improving oyster farming in the German North Sea. In 1880, our results show that the number of collected oysters is with 13 records per decade at its highest (Figs. 8A and 8B). Towards 1900, the numbers of live oysters are declining rapidly (Figs. 8A and 8B). This result is reflected by the literature stating that 4 – 5 million oysters were fished on the German island of Sylt in the middle of the 19th century, before fishing had to be stopped in 1882 because of low catch rates (Schümer, 1990). In Germany, the decline of *O. edulis* became especially apparent when other oyster beds on the East Frisian Islands were overfished by 1855 and fishing was no longer possible as well (Möbius, 1877).

At the beginning of the 20th century, no live oysters were found in the shallow water oyster beds in the North Sea (Fig. 5C). The only specimens of *O. edulis* found alive were collected by the 'Commission of the Scientific Investigation of the German Seas' from depth of 30 to 78 in the North Sea between the German island Helgoland and the Danish sand bank Horns Rev. These individuals were partly covered by barnacles and young oyster spat indicating to belong to healthy oyster beds. Ac-

cording to literature, these offshore oyster beds were discovered in the middle of the 19th century and spread out over 21 000 km^2 (Berghahn and Ruth, 2005; Gercken and Schmidt, 2014; Möbius, 1877; O. T. Olsen, 1883). Soon after the discovery of the new and profitable oyster beds, commercial fishery exploited them within a century (Hagmeier and Kändler, 1927). Hagmeier and Kändler, 1927 (p. 70) state: "During the cruise with the research vessel 'Poseidon' in March observations were made of the oysterbeds 'Austerngrunde' in the North Sea. Dredging along half a sea mile was completely unsuccessful, hence several attempts have been made resulting in 42 oysters in total (. . .)".

In the 1930s, our results recorded only one record of live oysters, but the largest number of empty shells of *O. edulis* was documented at that time (Figs. 5D and 8B). At the same time, the number of *C. fornicata* increased rapidly up to 22 records per decade (Figs. 5D, 8A and 8B). Originally, *C. fornicata* first established itself in Ireland and England in 1870, but the museum collections recorded the first individual 60 years later (Blanchard, 1997). This pattern of rapid spread and a subsequently stable level is typical for neozoan species that adapt to a new environment and can be observed in many invasive species (Geburzi and McCarthy, 2018; Herborg et al., 2003; D. Thieltges et al., 2003). Many neozoa pass unnoticed during the first stage of invasion, because the populations are small and localized after introduction ('lag-phase'). This may be an explanation why the museum collections have no records of *C. fornicata* before 1926, although the introduction date is known to be 1870 (Blanchard, 1997). Another explanation could be a lack of data in this study because not all European museums were included. For this study, only few large museums containing North Sea material were included that already digitalized their collections and made them public. Most museums only started recently to digitalize their collections and thus possible records of *C. fornicata* could have been documented but are not included in this data set.

The second stage of invasion, which often rises public awareness, is the expansion stage (Boudouresque et al., 2005; Geburzi and McCarthy, 2018; Gothland et al., 2014; Victoria, 2010). During this stage, the population is growing rapidly, which is also documented by the museum collections of *C. fornicata*. The subsequent persistence stage is distinguished by natural fluctuations of the population size, which can partly also be observed in *C. fornicata* (Fig. 8A). Here, the numbers decline after the rapid increase, which could also be an artefact of collection events. As Guralnick and Van Cleve, 2005 state, only outstanding or invasive species were collected to document the change in ecosystems. After the first 30 years of collection events, the excitement about the spread of the invasive *C. fornicata* may have passed and collection events declined thus the declining number of collection records. This assumption is supported by our results showing only one individual recorded for the last

decade, although *C. fornicata* is commonly found on the North Sea coasts nowadays (D. Thieltges, 2002; D. Thieltges et al., 2003).

The arrival of *C. fornicata* as an additional competitor could have been another burden for *O. edulis*, which could have accelerated the decline of the populations of *O. edulis*. The fishing industry feared *C. fornicata* to be harmful for the oyster beds, especially because they occurred in huge numbers soon after arrival (Fig. 8B) (D. Thieltges et al., 2003). Since the snail is a suspension feeder filtering phytoplankton and particulate organic matter as is the European flat oyster, it was assumed that *C. fornicata* could act as a feeding competitor, but this could not be verified (Blanchard, 1997; de Montaudouin et al., 1999; D. Thieltges et al., 2003; Thouzeau et al., 2000). It has also been shown that adult individuals of *C. fornicata* are able to ingest large particles such as *O. edulis* larvae, however, since *O. edulis* is also feeding on planktonic larvae and thus also on *C. fornicata* larvae, the predation effect is levelled out (Lapegue et al., 2006; Möbius, 1877; Pechenik et al., 2004). Another assumed risk represented by *C. fornicata* for oyster beds was that it would change the environment by massive production of pseudofaeces, enriching the soft sediments in oyster beds (Ehrhold et al., 1998; D. Thieltges et al., 2003).

On the contrary, it would also be possible that the dying oysters gave room for the common limpet slipper to spread, because the oysters were strong competitors. This hypothesis would be supported by our results that illustrate that the population of *O. edulis* was already endangered when the numbers of *C. fornicata* are increasing rapidly (Fig. 8A).

After most oyster beds went extinct in the 1940s, only one population of *O. edulis* survived in the Limfjord (Denmark) (Gercken and Schmidt, 2014). The results recorded some individuals found alive in the Limfjord in December 1869, but mostly empty shells were washed ashore in the middle of the 20th century. Literature states that the population of *O. edulis* in the Limfjord started to grow after a storm in 1825 connected the previously isolated Limfjord with the North Sea (Gercken and Schmidt, 2014). After a few decades the population was big enough for commercial fishing. Despite being fished the population is still thriving nowadays (Gercken and Schmidt, 2014).

Thus, we cannot confirm that the European flat oyster became extinct in the North Sea in the 1940s. Individual specimens can still be found in the North Sea (Gercken and Schmidt, 2014; Yonge, 1960). This is also supported by recent observations in off shore wind parks in Horns Rev (Denmark) and near Egmond aan Zee in the Netherlands (Bouma, 2012; Vattenfall, Skov-og et al., 2006). They were established in 2002 and 2006 respectively. In both cases, *O. edulis* was found in the intertidal zone on the monopiles of the offshore wind parks. The museum collections documented the presence of the oyster in the past in both areas, so it can be con-

cluded that oyster spat from remaining oyster beds settled down on this new habitat. Because of these new findings, we support the results of habitat controls that conclude that *O. edulis* is found very rarely and is threatened by extinction rather than being extinct (Rachor et al., 2013; Zettler et al., 2018).

The results of this study show the importance and scientific potential of museum collections. Combining thorough collection documentation and existing public collection databases provided a unique basis to reconstruct the historical distribution of *O. edulis* and *C. fornicata*. With this dataset, the spread of the invasive species *C. fornicata* and the decline of the native species *O. edulis* could be reconstructed geographically from the 1820s to the year 2018. For the future, it would be helpful to have a public database combining the museum collections for further studies about *O. edulis, C. fornicata* or future studies about other North Sea species, since those data bases provide valuable historical facts.

Conclusion

Our study reveals the value of natural history collections. By combining public records of Natural History museum all over Europe and records from the collections of small museums in Northern Germany (NORe e.V museums), we were able to reconstruct the historical distribution of *O. edulis* in the North Sea from the 1820s to the year 2018. Furthermore, we could reconstruct the process of invasion of *C. fornicata* in Northern Europe, which does not take place until *O. edulis* records of live shells had already declined dramatically and the records for dead shells increased. Our data suggest that the common limpet slipper is not responsible for the near extinction of *O. edulis* in the North Sea, since the numbers of *C. fornicata* exploded not until the 1940s – ten years after the local extinction of *O. edulis* in the North Sea.

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Chapter 2

Phylogeography in an "oyster" shell

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Abstract

By using ancient DNA methods on dry shells from museum collections, the historical phylogeography of *Ostrea edulis* was successfully depicted in its native range for the first time. This research determines to reconstruct the historical population structure of the European flat oyster across Europe in the 1870s – including the now extinct Wadden Sea. The historical phylogeography of the European flat oyster has been shaped not only by anthropogenic influences, but also by natural dispersal ability and historical barriers to gene flow. In total, four haplogroups have been reconstructed with one haplogroup having a patchy distribution from the North Sea to the Atlantic coast of France. This irregular distribution could be the result of translocations. The other three haplogroups are restricted to geographic ranges, which indicates adaptation to local environmental conditions or geographical bar-

riers. They could be founder effect populations after long distance dispersals of oysters that were constrained in their distribution to northern and southern glacial refugia in the past. The comparison with present-day studies on *O. edulis* revealed a similar population structure that indicates a temporally stable pattern over the past 150 years despite large-scale translocations. The advantage of this historical phylogeographic study in comparison to present-day investigations was the discovery of an autochthonous population in the German and Danish Wadden Sea in the late 19th century, where *O. edulis* is extinct today. This result provides a possible explanation for the extinction of the European flat oyster in the Wadden Sea and for its constant absence until today.

Introduction

Today, many plant and animal species are threatened or endangered by extinction, mainly due to human influences. The anthropogenically driven species decline exceeds the natural rate (Rosser and Mainka, 2002). Oysters are prominent examples of endangered species as a result of overexploitation and disease (Beck et al., 2011; Gercken and Schmidt, 2014). The European flat oyster *Ostrea edulis* Linnaeus, 1758 is native to the shallow regions of the Atlantic coasts from Norway to North East Africa as well as from the Mediterranean Sea to the Black Sea (Lapegue et al., 2006; Strauch and Thüry, 1985; Yonge, 1960). The oyster was very common until the 19th century, but the population sizes eventually declined, at least in part due to overfishing (Gercken and Schmidt, 2014). Subsequently, large translocation efforts were undertaken to sustain the local oyster beds (Bromley et al., 2016; Yonge, 1960). These translocation efforts were unsuccessful in the Wadden Sea, where *O. edulis* went locally extinct in the 1930s. The decline in the remaining range was further accelerated by disease outbreaks in the late 1970s and early 1980s (Abollo et al., 2008; Gercken and Schmidt, 2014; Hayer et al., 2019; Laing et al., 2006).

Given overfishing and translocations, it seems probable that the distribution and genetic structure of the European flat oyster have been shaped not only by human actions, but also by natural dispersal ability and historical barriers to gene flow. The phylogeography of marine species in Europe appears to be strongly influenced by the Last Glacial Maximum about 10 000 years ago (Fratini et al., 2005; Maggs et al., 2008). In many marine species several periglacial refugia have been reconstructed, from where the European coasts were recolonized (Maggs et al., 2008). The current phylogeography of the European flat oyster suggests that populations from the North Sea, the Atlantic and the Mediterranean Sea together with the Black Sea are genetically distinct (Diaz-Almela et al., 2004; Lallias et al., 2010). The now extinct

Wadden Sea population could not be included into those studies of present day genetic diversity, which may provide vital clues for the survival of oysters at large.

Human activities and their consequences have certainly played a role in the massive decline in wild *O. edulis* populations, but the reasons for the complete extinction of the Wadden Sea population remain poorly understood (Hayer et al., 2019; Laing et al., 2006). This highlights a general problem in conservation biology: as soon as a species or population goes extinct, it becomes difficult to reconstruct the reasons for their demise. Maladaptations to rapid environmental changes and upcoming diseases are one reason for extinctions (Pandolfi and Kiessling, 2014; K. F. Smith et al., 2006). Alternatively, populations become too small to be self-sustaining (McKinney, 1997). To answer if a species has disappeared due to small population size or arising maladaptations in the face of global change, sequencing historical specimens from museum collections with ancient DNA techniques offers the only possibility to analyse an extinct species or population genetically (Hung et al., 2013; Oswald et al., 2019; Suarez and Tsutsui, 2004). Only in the last two decades has DNA of mollusc shells been successfully extracted and sequenced, which encouraged us to analyse museum collections of dry shells of *O. edulis* (Der Sarkissian et al., 2017; Doherty and Was, 2007; Geist et al., 2008).

This study aims to reconstruct the historical phylogeography of *O. edulis* in its native range to determine historical population structure of the European flat oyster by applying aDNA methods on a unique collection of European oyster shells collected in the 1870s from across Europe, including the now extinct Wadden Sea.

Material & Methods

Mitochondrial genome assembly of modern oyster

To map historical DNA sequences accurately, we generated a reference mitochondrial genome from a freshly collected individual of *O. edulis*, which was purchased at the Limfjord (Denmark) in October 2018. Modern DNA extraction was performed in the IKMB in Kiel (Germany). 25 mg of soft tissue were extracted with the MagAttract HMW DNAKit (Qiagen) following manufacturers protocol and fragment length was measured using Agilent TapeStation 4200. A Chromium library (10x Genomics; Zheng et al., 2016) was prepared and sequenced on one Illumina HiSeq4000 lane. After sequencing, 10x barcodes were removed from reads using Trimmomatic v0.33, specifying HEADCROP:23 for forward reads and HEADCROP:1 for reverse reads (Bolger et al., 2014). Trimmed reads were used as input for MitoZ with the option "--genetic_code 5" to generate the mitochondrial assembly (Meng et al., 2019). The assembly was compared with the existing mitochondrial genome

Figure 9: Macroscopic photography of both shells of one individual of *Ostrea edulis*, belonging to the collection of the Zoological museum Kiel. The left shell has a drill hole of approximately 8mm in diameter underneath the ligament (circled). The right shell is undamaged.

(GenBank acc. no. JF274008) in Geneious v. 2020.0.4 (Kearse et al., 2012). The new mitochondrial genome was uploaded to Genbank with the accession number MT663266.

Preparation of historical oyster shells

We used museum collection material to generate the historical DNA sequences (see supplementary Table S7). Shell material stems from the historical oyster collection of the Zoological Museum in Kiel, Germany, which is invaluable since it is composed of *O. edulis* sampled along all coasts of Northern Europe between 1868 to 1885, which is prior to their decline (Möbius, 1877).

Shells of historical *O. edulis* were bleached, rinsed with distilled water and dried overnight. Using a Dremel hand drill with a round dental drill attachment, the uppermost layer was first removed to exclude any epibiota before a hole was drilled into the inner surface of the shell (see Fig. 9). Generally, the inner side of the shells was used for drilling. Drillings were collected on a weighing paper and transferred into a 2 ml tube. To avoid cross-contamination a new drill bit was used for each sample and the weighing paper was also changed.

Ancient DNA extraction and sequencing

DNA extraction and pre-PCR steps were performed in clean room facilities dedicated to aDNA research, which were never used for fresh oyster material. DNA extraction was performed with approximately 150 mg of shell material following a silica-based protocol with 0.5% SDS added to the extraction buffer to bind the calcium of the shells (Dabney et al., 2013; Der Sarkissian et al., 2017; Krause-Kyora et al., 2018). Negative controls for all experimental steps were included to rule out contamination. From each sample, double-stranded DNA sequencing libraries that were partially treated with uracil DNA glycosylase (UDGhalf) were prepared according to an established protocol for multiplex high-throughput sequencing (Rohland et al., 2015). Sample-specific indices were added to both library adapters via amplification with two index primers. Extraction and library blanks were treated in the same manner. The libraries were sequenced on 1/50 of a lane on the Illumina HiSeq 4000 platform (2*75 cycles) in the same Sequencing Centre following the manufacturers' protocol.

Bioinformatics

De-multiplexing was performed by sorting reads corresponding to their p7 and p5 combinations using the Bcl2fastq software (Illumina, Inc.). All generated sequences were processed according to published protocols specific for aDNA using the EAGER pipeline (Peltzer et al., 2016). We mapped all reads against the newly assembled mitochondrial reference genome of *O. edulis* (Genbank acc. no. MT663266) using the Circular mapper module of the EAGER pipeline with the default setting for aDNA reads (Danic-Tchaleu et al., 2011; Peltzer et al., 2016). All duplicate reads were removed applying DeDup version 0.12.2, part of the EAGER pipeline, with the default options (Peltzer et al., 2016). To verify aDNA data sets, we evaluated the presence of postmortem DNA damage signatures from read alignments using mapDamage version 2.0.8 (Jónsson et al., 2013). Consensus sequences were generated applying the vcf2genome script.

Phylogenetic reconstruction

For phylogenetic reconstruction, we aligned all sequences to the complete mitochondrial genome sequence of *O. edulis* we assembled (see first section). We rooted the phylogenies with two outgroups, *Ostrea lurida* and *Ostrea denselamellosa* (acc. nos. KC768038, NC_015231, Xiao et al., 2015; Yu and Li, 2011) from NCBI GenBank.

In order to keep as many sequences as possible, but to build a robust phylogeny at the same time, we extracted the single nucleotide polymorphisms (SNPs) of

each sequence based on its VCF file and calculated the percentage of unidentified SNPs per sequence. Based on this calculation, we reconstructed phylogenies with all sequences that fell below a certain percentage of missing SNP's. We compared phylogenies with more than 95% to more than 60% of SNPs missing in all sequences in 5% steps. This was tested by calculating Neighbour-Joining trees applying the Tamura-Nei Genetic Distance Model and 100 bootstrap resamples in Geneious. The tests resulted in a robust phylogeny based on sequences that had no more than 90% unidentified SNPs.

After the initial assessment of the effect of missing data on the phylogenetic reconstruction, we reconstructed a maximum likelihood phylogeny using the General Time Reversible model of complete mitochondrial genomes in MEGA X (Stecher et al., 2020). We used all sites and estimated the transition/transversion ratio. Branch support was calculated from 500 bootstrap replicates. The branches of all phylogenies were collapsed when the bootstrap support was under 35 using TreeGraph2 (Stöver and Müller, 2010). The haplogroups were primarily selected by a number of diagnostic SNPs that are shared by sequences and are specific for a haplogroup and secondarily by bootstrap support.

To assess how different historical and current sequences are, we built a second maximum likelihood phylogeny with the same settings, but this time using only a fragment of the mitochondrial gene encoding for the 12S rRNA. For this fragment, modern population genetic data exists in NCBI Genbank (acc. nos. AY157516 to AY157529, HQ259072 and JQ611471) (Diaz-Almela et al., 2004; Malkowsky and Klussmann-Kolb, 2012). Historical sequences without identified nucleotides within the 12S rRNA encoding gene were automatically removed.

Population genetic analyses

All population genetic analyses were conducted in R version 3.6.2 (R. C. Team et al., 2019). Given the fact that several of the sequences contained missing sites, which cannot be accounted for in several population genetic analyses, the alignment was amended for the use of statistical analyses. For every haplogroup, we extracted one sequence with the highest coverage quality and multiplied it with the number of individuals present in the respective haplogroup. This is a conservative approach relying on the assumption that the same SNPs would be present in all the individuals of each haplogroup without any diversity within each haplogroup.

The genetic differentiation between all population pairs was calculated as Nei's G_{ST} and Jost's D with the functions 'pairwise_Gst_Nei' and 'pairwise_D' of the 'mmod' package (Winter, 2012). G_{ST} is a derivative of the classical fixation index F_{ST}, developed for data with more than two alleles at a locus (Hahn, 2019). We

Table 3: comparison of the mitochondrial genomes of *O. edulis* in total and in the COI region.

	JF274008 / MT663266	
	total	**COI**
Number of nucleotides	16323/16356	1566/1596
Nucleotide difference [%]	0.22	1.9
Number of SNPs	385	81
SNPs [%]	2.4	5.1

estimated significant deviations from zero (no differentiation between population pairs) by comparison with an empirical distribution of G_{ST} values based on 1000 permutations (Jost'D calculation in supplementary Table S8).

Results

We assembled a new complete mitochondrial genome of *O. edulis* (GenBank acc. no. MT663266) that differs from the existing mitochondrial genome of *O. edulis* in GenBank (acc. no. JF274008) in the COI region (see table 3). It is with 16 356 nucleotides 36 bp longer than the existing mitochondrial genome (JF274008) and encodes 38 genes, including twelve protein-coding genes (PCGs), three rRNAs and 23 tRNAs on the same strand (Fig. 10).

The reference genome (JF274008) misses 36 bp in the COI gene, which resulted in an error removing many reads from the aDNA sequences while mapping against it. Whilst mapping the aDNA reads against the new genome (MT663266), the aligning problems in the COI gene region disappeared. We controlled the new alignment with the genome available in GenBank (JF274008) to exclude any sequencing or assembling errors.

We sampled 170 *O. edulis* shells, but only 75 samples contained DNA and were sequenced using Illumina HiSeq. 34 sequences could not be used for the phylogeny due to low coverage quality, while four sequences were removed because they date into the 20^{th} century. The final alignment is composed of 37 ancient mitochondrial sequences of *O. edulis*, plus both reference genomes and two outgroups (see supplementary Table S7). The historical genomes are submitted to GenBank, but have not yet been assigned an accession number.

Phylogenetic reconstruction and population genetic analyses

Based on the phylogenetic reconstruction of the complete mitochondrial genome we identified four monophyletic haplogroups, labelled according to their geographic

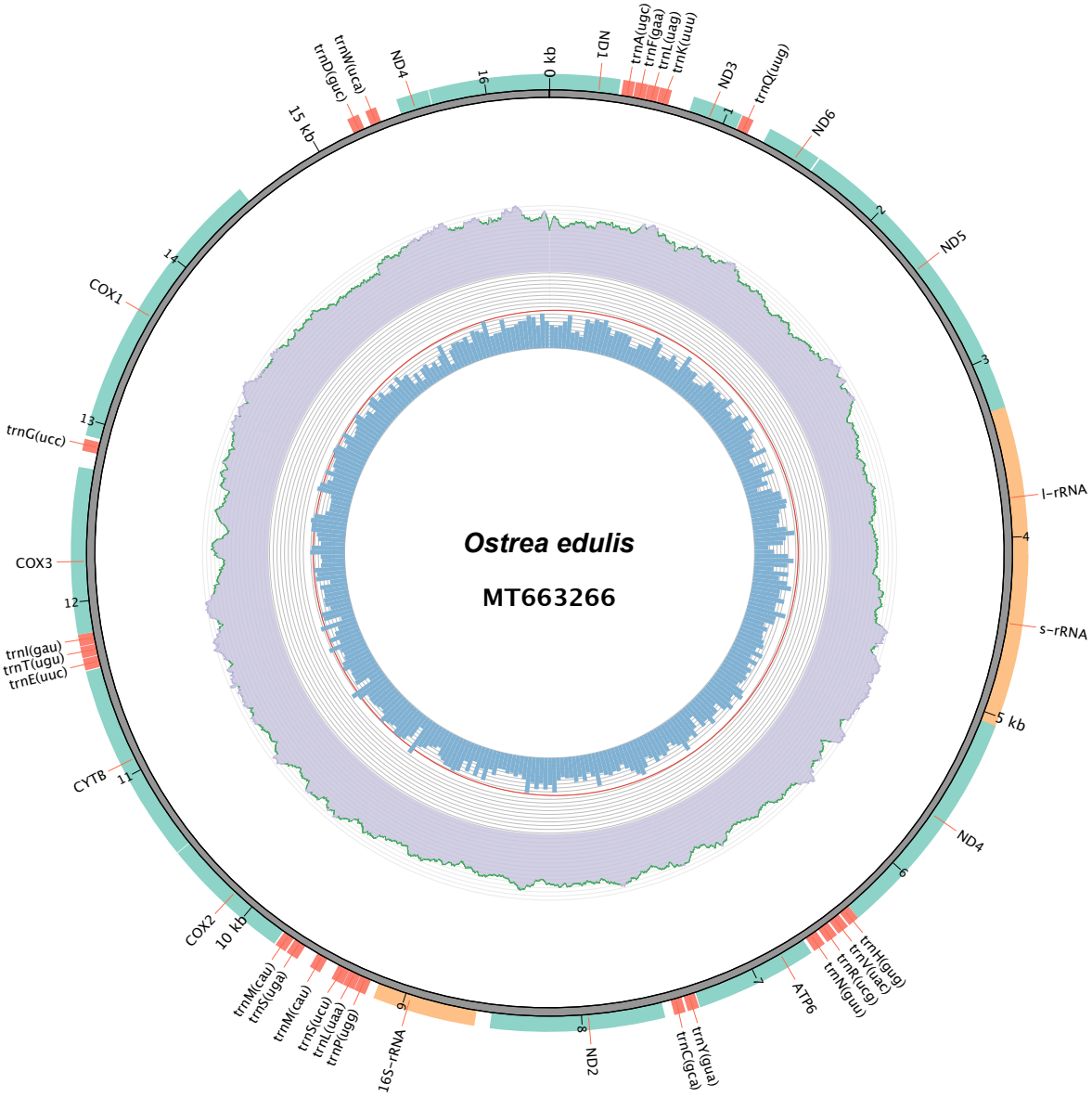

Figure 10: Mitochondrial genome map of *Ostrea edulis* drawn with MitoZ. The innermost circle shows the GC content, the middle circle reveals the depth distribution with a green outline when the depth is larger than the upper quartile. The outermost circle shows the gene features for proteins (green) and rRNAs (yellow) that are abbreviated with standard abbreviations. The one letter amino acid code is used for tRNA (red) designation.

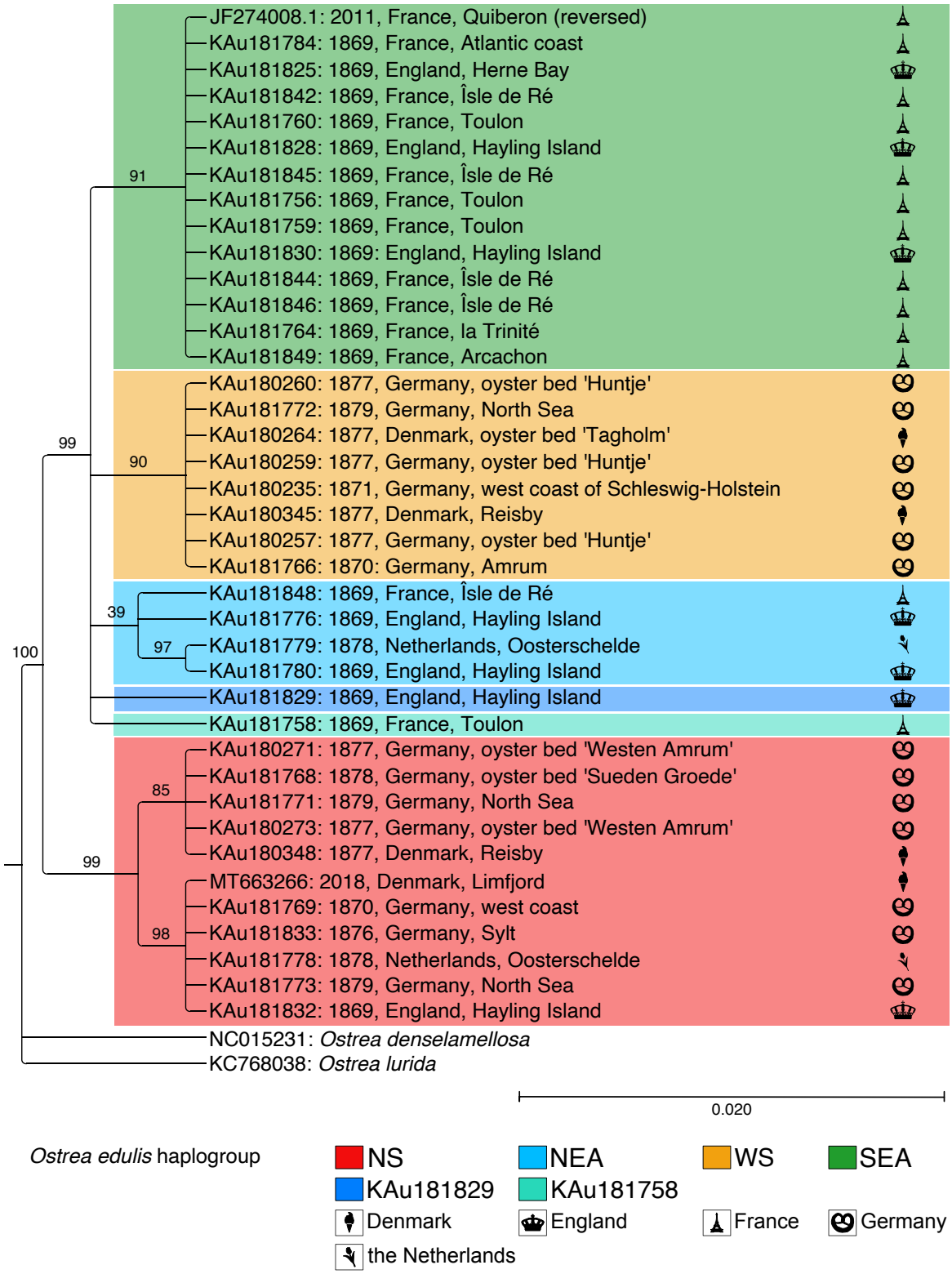

Figure 11: Phylogeny of ancient mitochondrial genomes mapped against MT663266 (complete mitochondrial genome generated in this study) and controlled with JF274008.1 (complete mitochondrial genome in Genbank) using the maximum likelihood method and General time Reversible model using all sites. Bootstrap node support (in percent, from 500 replicates) is shown next to the branches. All branches with less than 35 bootstrap support are collapsed. Colour shading highlights different haplogroups. Phylogeny is rooted with *O. lurida* and *O. denselamellosa*. This analysis involved 41 nucleotide sequences with a total of 16 653 positions in the final dataset.

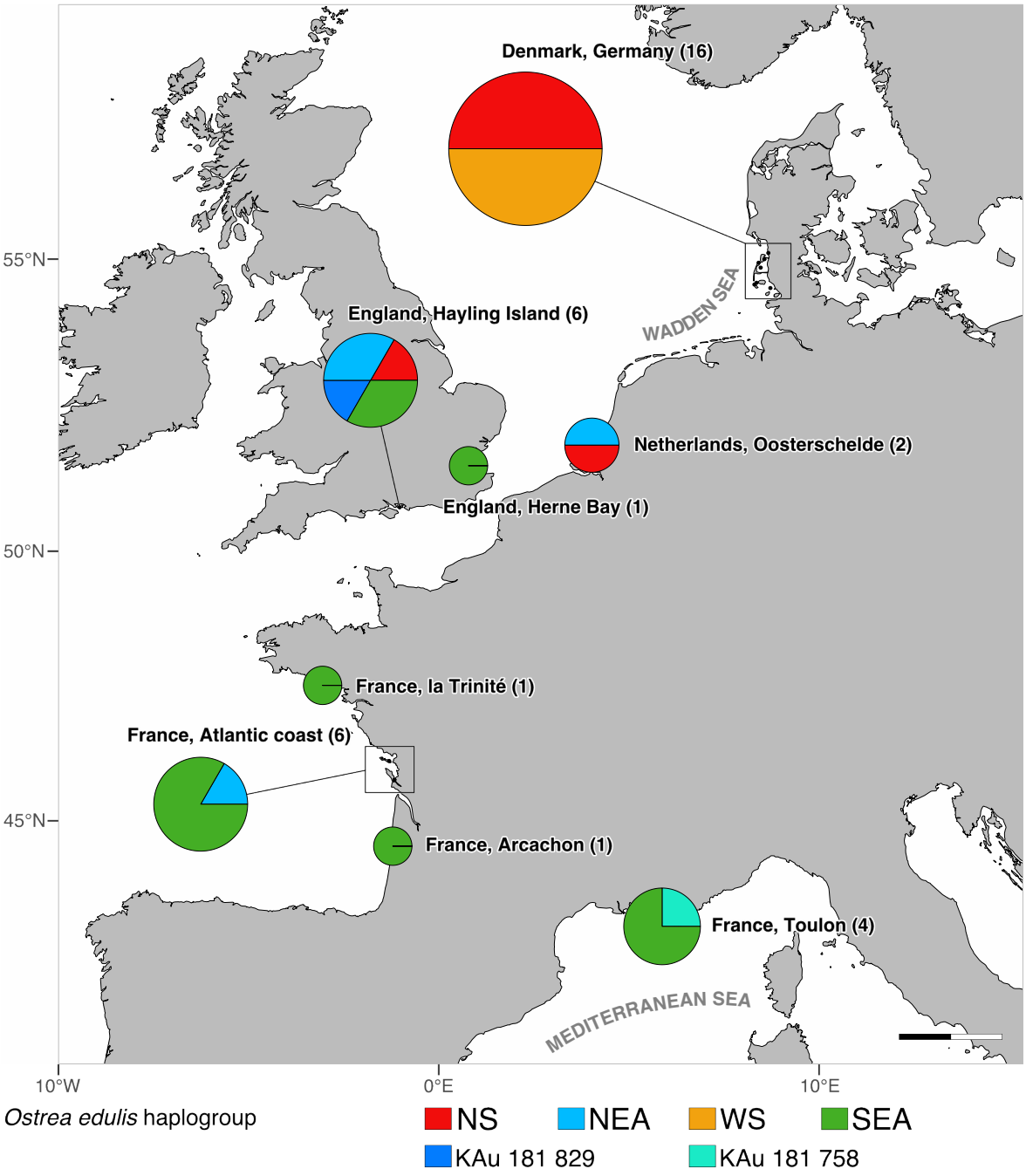

Figure 12: Geographic distribution of mitochondrial haplogroup frequencies of *Ostrea edulis* in Europe between 1869 and 1879. The smallest pie chart represents a single individual. All sample sizes are given in brackets on each location. Scale bar = 100km.

range as Wadden Sea (WS), North Sea (NS), North-East Atlantic (NEA) and South-East Atlantic (SEA) (see Figs. 11 and 12). They are defined by a number of diagnostic SNPs that are in this composition unique for a haplogroup (see supplementary Table S9). Haplogroup WS is based on eight sequences collected only along the Wadden Sea of Germany and Denmark, which is the most restrict distribution range of all haplogroups (Figs. 11 and 12). Haplogroup NS is a separate basal clade of eleven sequences that can only be found at the North Sea coasts. It is composed of two branches: One branch is composed of more local sequences that were collected at the North Frisian and Danish Wadden Sea islands and circumjacent oyster beds. The second branch includes sequences from all coasts of the North Sea like the Netherlands, England and Germany. Our new reference also falls into that branch (Figs. 11 and 12). Haplogroup SEA is composed of 13 individuals collected at the Atlantic and Mediterranean coasts of France and the South coast of England in 1869 (Figs. 11 and 12). The mitochondrial genome from GenBank, which originates from an oyster collected at the French Atlantic coast, also belongs to this haplogroup. Haplogroup NEA holds four aDNA sequences from the coasts of France, the Netherlands and England between 1869 and 1878 (Figs. 11 and 12). This haplogroup is composed of sequences that have many variable SNPs, and given more data, we might have split this haplogroup up in two haplogroups. Two sequences from Hayling Island, England and Toulon, France were not assigned to any haplogroup.

In comparison to the phylogeny based on the complete mitochondrial genome (Fig. 11), the haplogroups could not be resolved in the 12S rRNA phylogeny (see supplementary Fig. S3). The phylogeny is also poorly supported by bootstrap values, which makes it difficult to draw conclusions about a relationship between aDNA samples and formerly defined haplotypes (Diaz-Almela et al., 2004; Malkowsky and Klussmann-Kolb, 2012).

In order to identify the genetic differentiation between populations, calculations of Nei's G_{ST} were conducted between populations with four or more individuals and ranged from 0 to 0.343 (Table 4). Since the number of individuals per sampling site was sometimes only one or two, we combined several of the sampling locations: all specimens from England were summarized, as were the individuals collected along the French Atlantic coast, and German and Danish individuals were condensed to one population called "Wadden Sea". The Netherlands were excluded from the statistical calculations, since the number of individuals was very small. The model revealed partly a significant population structure across Europe (Table 4). The Wadden Sea is most distinct from the rest of Europe (Table 4). The populations from England are similar to those of the Mediterranean and the Atlantic coast of France, but distinct from the Wadden Sea (Table 4). The Atlantic and Mediterranean coasts of France are similar to each other, given that they mostly share the same

Table 4: Results of Nei's G_{ST} pairwise analyses of *O. edulis* populations from sampling sites with more than four sampled individuals. Pairwise p-values are shown in the top triangle (blue font). Values with significance are bolded (p-value < 0.05).

	Wadden Sea	**England**	**Atlantic**	**Mediterranean**
Sample size	20	7	8	4
Haplogroups	NS, WS	NS, NEA, KAu181829, SEA	NEA, SEA	KAu181758, SEA
Nei's G_{ST}				
Wadden Sea	-	**0.043**	**0.004**	**0.021**
England	**0.13678430**	-	0.163	0.473
Atlantic	**0.34252229**	0.06331849	-	0.472
Mediterranean	**0.31563499**	0.04836342	0.02782109	-

haplogroup (see Table 4).

Discussion

In the course of this study, we reconstructed 37 complete historical mitochondrial genomes extracted from dry shells of *O. edulis* and assembled a new high-quality mitochondrial genome from an European flat oyster originating from the Danish Limfjord in 2018. Danic-Tchaleu et al., 2011 assembled also a mitochondrial genome using long range PCR and sanger sequencing (Danic-Tchaleu et al., 2011). Because of the methodological differences, the extra 36 bp in our genome could be explained. With these references and complete mitochondrial aDNA genome data, we successfully reconstructed for the first time the phylogeography of *O. edulis* throughout the native range from the 1870s. The phylogeographic reconstruction revealed the presence of an autochthonous population in the German and Danish Wadden Sea where the oyster is extinct today. This finding provides a possible reason for the extinction of the European oyster in the Wadden Sea and for its continuous absence until today.

Present-day studies used 12S rRNA sequences of *O. edulis* to successfully resolve a phylogeographic pattern that is also supported by microsatellite studies (Diaz-Almela et al., 2004; Lallias et al., 2010). When performing a phylogeographic study on modern samples of *O. edulis*, sequencing the 286 base pairs region is a fast and cost-efficient solution, but the same does not apply to historical samples. The DNA of historical samples is highly fragmented and specific regions such as this specific fragment of the 12S rRNA may not be covered. To analyse as many single nucleotide polymorphisms (SNPs) as possible, which resolve the distribution pattern, we performed the more laborious shotgun sequencing to assemble complete mitochondrial genomes.

The oysters used in this study were collected by Karl August Möbius between 1869 and 1879, who was professor of Zoology and director of the Zoological museum in Kiel (Germany) at the time. Möbius received a research contract from the Prussian King and German Emperor Wilhelm I. in 1868 to investigate the possibility of artificial oyster farming in the German North Sea. Thus, he travelled all over Europe to sample European flat oysters and brought many specimens back to Kiel (Möbius, 1877). At the end of the 19th century, the anthropogenic influence on the oyster beds by exchanging oyster spat all over Europe was already common to sustain the oyster beds (Bromley et al., 2016; Gercken and Schmidt, 2014; Yonge, 1960). However, the haplogroups in our study do not reflect these massive translocations and are restricted to geographical ranges with the exception of haplogroup NEA. The patchy distribution of this haplogroup (see Fig. 12) could indeed be the result of translocations. Fullarton, 1891 stated that by the 1800s "most of the English natives are born in France or Holland, and are fattened at Whitstable or other beds in the South of England" (Fullarton, 1891). Bromley et al., 2016 pictured that imports to England originated from France, Scotland, the Netherlands, Ireland and other unknown source countries (Bromley et al., 2016). However, we could not include any oysters from the French coast of the English channel or other locations of Great Britain or Ireland to further confirm these hypotheses.

A conspicuous feature of our phylogenetic reconstruction is the split between the most basal haplogroup NS and the remaining lineages. This relatively old split could have formed when oysters were constrained in their distribution to two different glacial refugia. The basal haplogroup NS is widely spread in the central North Sea with the most western record in the English Channel, but no individuals with this haplotype were retrieved further south (see Fig. 12). It represents therefore a private haplogroup to the North Sea, which is indicative of a northern glacial refugium (Maggs et al., 2008). The Hurd Deep, a deeper trench in the western English Channel, and some ice free coasts along the North Sea could have been glacial refugia to different seaweed species (Coyer et al., 2003; Hoarau et al., 2007; Provan et al., 2005; Stam et al., 2001), molluscs (Luttikhuizen et al., 2003; Tarnowska et al., 2012) and decapods (Roman and Palumbi, 2004). Curiously, this northern lineage has not expanded further south after the glaciers retracted. Larval dispersal may be constrained by the internal counter clockwise circulation currents in the North Sea (Brown et al., 1999). It would also be possible that this northern lineage is not well-adapted to southern environmental conditions such as high temperatures.

The southern clade presents with three haplogroups a higher phylogenetic diversity than the northern clade. It is distributed along the East Atlantic coast and the Mediterranean Sea. We propose that this clade had one or more southern glacial refugia, and expanded northwards as the climate warmed. The presence of

several monophyletic groupings within this clade could be due to differentiation processes during its northward spread. The northwards expansion could have led to a series of founder effects presenting the different haplogroups restricted to a geographic range. This recolonization of Northern Europe from southern glacial refugia is common in both marine and terrestrial species (Andrew King and Ferris, 1998; Demesure et al., 1996; Hewitt, 1999, 2004; Hoarau et al., 2007; Provan and Bennett, 2008; Wallis and Arntzen, 1989).

Based on our data, the Atlantic and Mediterranean populations are not significantly different from each other, as might have been expected given the biogeographic break between them (see haplogroup SEA, Fig. 12). Phylogeographic studies consistently separate the Atlantic from the Mediterranean Sea not only in the European oyster (Diaz-Almela et al., 2004), but also in a diverse array of marine organisms such as marine algae and seagrasses (Coyer et al., 2003; J. L. Olsen et al., 2004; Provan et al., 2005), different invertebrates (Baus et al., 2005; Duran et al., 2004; Wilke and Pfenninger, 2002; Zane et al., 2000), bony fishes (Borsa et al., 1997; Cimmaruta et al., 2005; Gysels et al., 2004; Nakadate et al., 2005) and cartilaginous fishes (Chevolot et al., 2007). The missing genetic differentiation between the historical Atlantic and Mediterranean populations of *O. edulis* is likely an artefact of the low sample size – we were only able to sequence four individuals successfully – in the Mediterranean Sea. Moreover, all sequenced individuals were collected from a single location in the Mediterranean Sea.

Present-day phylogeographical studies identified not only significant differentiation between the Atlantic and Mediterranean (Diaz-Almela et al., 2004), but they as well as our historical data found a differentiated clade in the North Sea (Diaz-Almela et al., 2004; Lallias et al., 2010; Vera et al., 2016). Since all four studies found the same phylogeographic pattern, we conclude that this seems to be a temporally stable pattern over the past 150 years despite large-scale translocations. Additionally, our reference genome originating from the Limfjord (Denmark) in 2018 confirms that haplogroup NS still exists today (see Fig. 11). The Limfjordian oyster beds are a special case concerning the survival of natural beds of the European flat oyster, since it is the only surviving natural oyster bed in the North Sea. The Limfjord oyster beds are comparatively young to the oyster beds along the North Sea coast. Initially a brackish water body, the Limfjord became marine when a heavy storm connected it to the North Sea in 1825 (Yonge, 1960). Soon after, the first oyster beds were discovered in the Limfjord (Yonge, 1960). The Limfjordian oyster beds were, however, also supported by translocations of 18 million adult oysters originating from the Netherlands (Bromley et al., 2016). Our genetic results only verify the survival of haplogroup NS in the Limfjord, but cannot differentiate if this haplogroup came into the Limfjord via natural dispersal or translocation.

The most astonishing result is the autochthonous haplogroup WS, which is based on eight individuals that were without exception collected from the near shore of the Danish and German Wadden Sea, where the European oyster went extinct in the 1930s. It is the most northern haplogroup of the southern clade. Its restriction to the Wadden Sea may be by local adaptation to the distinct environmental conditions of the Wadden Sea. The oyster beds in the Wadden Sea were situated in deeper trenches between the mudflats, so that they were still covered with water during low tide (Möbius, 1877). But due to the tidal changes that expose the mudflats during low tide, the changes in temperature, currents and salinity in the deeper trenches are more extreme than in the central North Sea or the Atlantic coast. While haplogroup WS may have been well-adapted to the extreme environmental conditions of the Wadden Sea, it may not have been able to react to broader ecological changes and might have been particularly vulnerable to diseases, high temperatures, pollution or other global change threats. This possible vulnerability to ecological changes in combination with overfishing could have led to the extinction event of haplogroup WS in the early 20th century. This would also explain why *O. edulis* has not recovered yet in the Wadden Sea.

Nevertheless, it remains a mystery why *O. edulis* has not been recovering in the Wadden Sea in the past 80 years, although haplogroup NS survived in the Limfjord and was also present in the Wadden Sea in the late 19th century. One explanation could be the different environmental conditions between the Wadden Sea and the Limfjord: The Limfjord is a shallow water body of seven metres in average and opens through the Thyborøn canal into the North Sea, which is a canal of 1km in width. This narrow connection to the North Sea leads to a low volume inflow of salt water into the Limfjord, which results in a lower salt water content towards the East and modified tidal ranges and currents. Due to these environmental conditions, the Limfjord is less disturbed and thus a safer location for oysters to grow. Because of the remote location combined with reduced water inflow and currents, pathogens and competitors like *Bonamia ostreae* and *Magallana gigas* entered the Limfjord later than they did at other locations in Europe (Freitas et al., 2019; Madsen and Thomassen, 2015). Another hypothesis is based on the results of Ronza et al., 2018: they showed that *O. edulis* has not developed any resistance to bonamiosis in the Limfjord yet. The presence of *B. ostreae* in the North Sea may therefore be a reason why the native oyster has not recolonized the Wadden Sea again (Ronza et al., 2018). In the past, it has been observed that *O. edulis* was able to survive cold climatic conditions over long periods, but *B. ostreae* was eliminated (Madsen et al., 2013). However, such strong winters are in the course of the global warming scarce, which makes it difficult for *O. edulis* to resettle the Wadden Sea, when climatic conditions favour the survival of bonamiosis. Hence, the environmental difference between

the remote Limfjord and the Wadden Sea, altered climate conditions and parasites could explain why *O. edulis* has not resettled the Wadden Sea again.

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Chapter 3

First indication of Japanese mitten crabs in Europe and cryptic genetic diversity of invasive Chinese mitten crabs

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Abstract

The Chinese mitten crab (*Eriocheir sinensis*) is a prominent aquatic invader with substantial negative economic and environmental impacts. The aim of the present study was to reevaluate the genetic diversity of mitten crabs throughout their native and invaded ranges based on publicly available sequence data, and assess if multiple introductions or rapid adaptation could be responsible for biologically divergent mitten crabs in Northern Europe. We assembled available genetic data of a fragment

of the mitochondrial cytochrome c oxidase subunit one gene (COI) for all species of the genus *Eriocheir*. We applied phylogenetic and population genetic analyses to compare native and invasive populations, and to identify possible source populations. The phylogenetic reconstruction revealed that five COI sequences from Europe, morphologically identified as Chinese mitten crab, actually belong to the Japanese mitten crab (*Eriocheir japonica*), representing the first indication of its presence in European waters. All other COI sequences from Europe could unambiguously be assigned to the Chinese mitten crab. In some Northern German populations of Chinese mitten crabs, genetic diversity was surprisingly high, due to seven unique haplotypes encoding several amino acid substitutions. This diversity may reflect a cryptic introduction from an unsampled native location, or rapid adaptation in the invaded range. Based on the genetic diversity shared between native and introduced range, Feiyunjiang, a tributary of the Yangtze River, emerges as a plausible source population for the original introduction of Chinese mitten crabs to Europe. This study highlights the complex and dynamic invasion processes of mitten crabs in Europe. We urge to further monitor mitten crab invasions using genetic tools.

Introduction

Species invasions have altered the global ecological landscape dramatically in the past centuries. Their impacts are exemplified by the Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards, 1853), one of the taxa included in the list of the "world's 100 worst invasive alien species" (Lowe et al., 2000). Native to Russia, China, Korea and Taiwan, it has been introduced to Europe at the beginning of the 20th century and subsequently to the United States via the ballast water of large shipping vessels (Clark et al., 1998; Dittel and Epifanio, 2009; Herborg et al., 2003, 2005; Peters, 1933b; Rudnick et al., 2000; Rudnick et al., 2003). The economic and ecological effects of its invasion are staggering. While declining in abundance in its native range, where it is considered a delicacy and farmed extensively (Yuan, 2005), it has become an unprecedented nuisance in its introduced range. During past mass occurrences, when thousands of crabs migrated from their adult inland freshwater habitats to marine spawning grounds, fishermen lost nets and even abandoned certain fishing grounds (Rudnick et al., 2000). River banks were destabilized due to the extensive burrowing activity of adult crabs (Rudnick et al., 2005). Moreover, ecological communities have the potential to be altered by competition with native crabs and crayfish (Dittel and Epifanio, 2009; Gilbey et al., 2008; Rudnick et al., 2000).

Identifying the source or sources of such widespread invasions is an import-

ant task for risk assessment and species management. Assigning the geographic sources of invasive populations requires geographic differentiation within the native range. Such differentiation allowed, for example, to pinpoint the source populations of introduced olive populations in Hawaii and Australia as South Africa and western or central Mediterranean, respectively (Besnard et al., 2007). In contrast, if native populations are genetically homogeneous, or the employed genetic markers are not variable enough to detect genetic structure, source populations can only be assigned to broad geographic regions. The European shore crab (*Carcinus maenas*), for example, has invaded the East coast of North America twice (Roman, 2006). The source of each invasion could be broadly categorized as Northern and Southern European, respectively, based on slight genetic structure in the native range and ecological differences of the two invading populations. More detailed assignment was hampered by broadly distributed common haplotypes of the studied marker in the native range, possibly caused by anthropogenic reshuffling of native diversity. In the case of another well-dated species invasion, the source of introduction of the North American spiny-cheek crayfish (*Faxonius limosus*) to Poland in 1890 could not be determined because the invaded range is dominated by a haplotype common throughout the native range (Filipová et al., 2011). Similarly, Hänfling et al., 2002 were unable to pinpoint a source population for the mitten crab invasion to Europe due to apparent genetic homogeneity in the native range. This could have been due to small sample sizes (six to 10 individuals sampled per population) and observed low diversity (five haplotypes only) of the employed genetic marker, a fragment of the mitochondrial cytochrome oxidase subunit one (COI). Under-sampling the native range is a general problem, not only with regard to the number of populations, but also with number of individuals analyzed per population (Muirhead et al., 2008). If only a few individuals are sampled, much of the genetic diversity present at any one site might be missed, obscuring assignment probabilities (Muirhead et al., 2008).

Since the initial assessment by Hänfling et al., 2002, several phylogeographic studies have characterized the genetic population structure of mitten crabs in their large native range (Sui et al., 2009; C. Wang et al., 2008; Xu et al., 2009). These studies detected five monophyletic lineages (Fig. 13). Some authors refer to these lineages as different species (e.g. X. Chen et al., 2017; Chu et al., 2003; Naser et al., 2012; C. Wang et al., 2008), others as subspecies or lineages (e.g. Tang et al., 2003; Xu et al., 2009; D. Zhang et al., 2012). We do not aim to resolve these taxonomic issues, but use the species names as unambiguous labels throughout the text. These lineages have distinct but partially overlapping ranges (Komai et al., 2006; Xu et al., 2009): the Hepu mitten crab (*Eriocheir hepuensis* Dai, 1991) is present in Southern China from Hepu to Oujiang, the Chinese mitten crab from Tongan in China to Vladivos-

tok in Russia including Korea, the Japanese mitten crab (*Eriocheir japonica* (De Haan, 1835)) is the only lineage present in Japan, but occurs in Russia and Korea as well, *Eriocheir ogasawaraensis* (Komai et al., 2006) is restricted to the Ogasawara Islands and an additional formally undescribed Japanese mitten crab lineage is endemic to Okinawa (Fig. 13) (C. Wang et al., 2008). Based on combined sequence data for two mitochondrial gene fragments, population structure was significant in Chinese and Japanese mitten crabs, but less so in the Hepu mitten crab (C. Wang et al., 2008) using cytochrome oxidase subunit two and cytochrome b as genetic markers; (Xu et al., 2009 using COI and cytochrome b). The significant population differentiation of both Japanese and Chinese mitten crab provides a working baseline for assigning source populations. To date, Japanese mitten crabs have sporadically occurred outside their native range in the United States only (Benson and Fuller, 2019; Jensen and Armstrong, 2004), but have not been detected in Europe yet. Chinese mitten crabs, on the other hand, have invaded both Europe and the United States successfully. Population genetic studies of Chinese mitten crabs from the invaded range came to quite discordant conclusions. Some studies found that populations within Europe were admixed (Czerniejewski et al., 2012; Hänfling et al., 2002), while others found significant levels of differentiation (Herborg et al., 2007; Otto, 2012). In contrast, only a single COI haplotype of this species has been reported from the United States (Hänfling et al., 2002). While the source populations remained obscure, Hänfling et al., 2002 identified three haplotypes that were shared between the native and invaded ranges as well as a widespread invasive haplotype unknown in the native range, but found in both Europe and the United States. Given the presence of both invasive haplotypes detected in the native range as well as invasive haplotypes not detected in the native range, they concluded that multiple invasions occurred in Europe, and that the USA were likely invaded via Europe. Invasive populations can become melting pots of novel genetic combinations with unforeseen adaptive potential (Geller et al., 2010). While species invasions are often associated with a loss of genetic diversity in the introduced range, either because only a few individuals invaded the range, and/or because genetic drift in these small populations subsequently reduces diversity (Nei et al., 1975), multiple invasions can alleviate the effects of these founding events (Dlugosch and Parker, 2008). The brown anole lizard (*Anolis sagrei*), for example, has invaded Florida multiple times from geographically distinct source populations. Each source population had a unique genetic makeup, and admixture in the invaded range led to genetic diversity higher than in populations of the native range (Kolbe et al., 2004, 2007). Similar observations have been made for the common ragweed (Genton et al., 2005). Each new invasion might bring in new genetic variation, accompanied by novel ecological and physiological strategies that warrant attention (Geller et al., 2010).

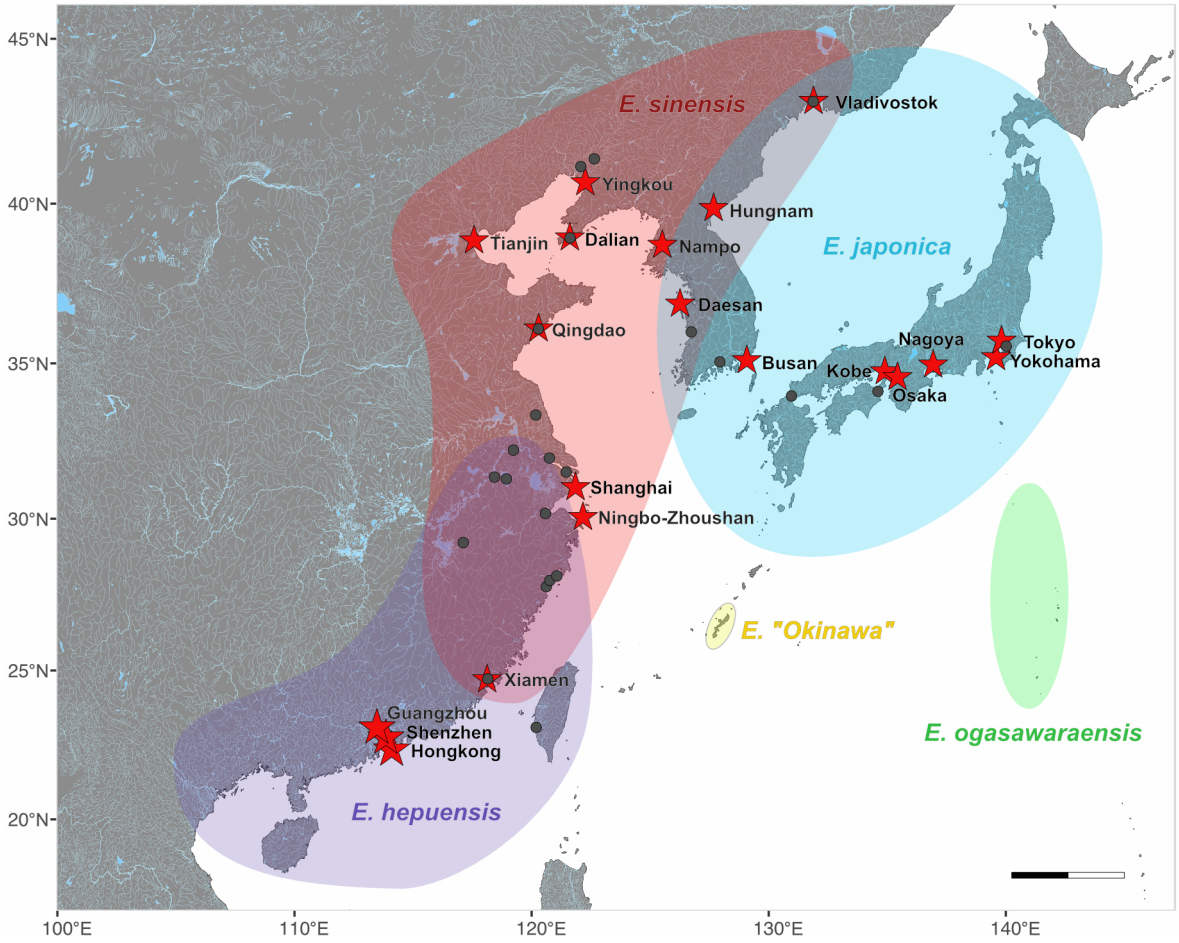

Figure 13: Native distribution of mitten crabs of the genus *Eriocheir*. Map redrawn from Xu et al., 2009, and species ranges interpolated around their sampling sites (marked by grey points) for each lineage to highlight range overlaps. Large shipping ports (marked by red asterisks) are most likely invasion sources.

This could be the case for Chinese mitten crabs. Otto, 2012 reported on a novel reproductive behavior and physiology in invasive mitten crabs. In contrast to earlier reports (Anger, 1991), mitten crabs completed their life cycle in the brackish Baltic Sea, and post-spawning females migrated back into the rivers, and did not die as in other populations. Otto, 2012 concluded that this novel behavior might have been caused by a cryptic invasion of mitten crabs with different physiological requirements, in line with an earlier conclusion of cryptic invasions based on genetic data (Hänfling et al., 2002). Both lines of evidence, however, allow for an alternative explanation: invasive mitten crabs could have adapted rapidly to the brackish water conditions of the Baltic Sea, leading to novel genetic, physiological and behavioral diversity.

Rapid adaptation is emerging as a common feature of species invasions (Card et al., 2018; Dlugosch and Parker, 2008; Prentis et al., 2008). In animals, hybridization between distinct introduced lineages, allele shifts due to bottlenecks and standing genetic variation are likely agents of rapid adaptation. Selection acts fast-

est on standing variation, and this process is suggested to be the most common driver of rapid adaptation (Prentis et al., 2008). The Burmese Python (*Python molurus bivittatus*), for example, is native to Southeast Asia and was introduced to Southern Florida in the early 1980s (Card et al., 2018). Despite several freezing periods that caused high python mortality, this species has become a successful invader into North America. Investigations showed that these freezing events resulted in a shift of python physiology caused by changes in allele frequencies in functional genes (Card et al., 2018).

The goal of this study was to re-evaluate the genetic diversity of mitten crabs of the genus *Eriocheir* throughout its native and invaded ranges, and to assess if multiple introductions or rapid adaptation might have caused the recent appearance of biologically distinct mitten crabs in Northern Europe. Given a large body of previous work, we utilized publicly available mitochondrial sequence data. We employed different approaches to assign source populations to the invasive populations in Europe and the United States. First, we reconstructed phylogenetic relationships among *Eriocheir* sequences, grouping thereby invasive individuals into the evolutionary lineages known from the native range. In the next step, we assessed the native distribution of haplotypes also present in the introduced range. Then, we calculated genetic distances for all population pairs, which we use on the one hand to evaluate population genetic structure in the native range, and on the other hand to identify which native populations are most similar to introduced populations. We assume thereby that allele frequencies have not shifted significantly since the invasion, and that enough individuals invaded the new range to mirror native allele frequencies. Bayesian assignment tests have been proposed as a suitable alternative to assign invasive individuals to source populations (Geller et al., 2010). They may not assume that populations are in migration-drift-equilibrium (Aktas, 2015), but are limited to detect very recent gene flow between populations within the past few generations (Herborg et al., 2007). Given that the initial invasion occurred around 30 generations ago (generation times taken from Dittel and Epifanio, 2009), neither the assumptions of genetic distance measures nor assignment tests are likely to be met. Thus, neither approach provides an ideal fit to the pattern of mitten crab invasion, but our inferences are reinforced when several approaches point to the same source populations. Lastly, we assessed the potential for adaptive evolution in the investigated mitochondrial DNA fragment by identifying amino acid substitution patterns in the introduced haplotypes. Mitochondrial genes are not known to be directly involved in osmoregulations or other adaptations to low-salinity conditions, but the amino acid sequence is highly conserved, being under strong purifying selection (Meiklejohn et al., 2007; Pentinsaari et al., 2016). Any changes in the amino acid sequence we detect are at least unusual and warrant further investigation. Based on

our findings, we form several hypotheses regarding the mitten crab invasions that should be followed up using expanded geographic sampling, genomic approaches and historical collections.

Methods

Data preparation

We downloaded all available sequences for the genus *Eriocheir* and for two outgroup species, *Neoeriocheir leptognathus* and *Platyeriocheir formosa* (acc. nos. AF316537, AF317326; Tang et al., 2003), from NCBI GenBank (http://www.ncbi.nlm.nih.gov/Genbank), and sequences of the Chinese mitten crab from the Barcode of Life Data System (BOLD, http://www.barcodinglife.org), ignoring sequences already available in GenBank. An initial screening showed that most sequences were mitochondrial. Thus, we mapped all sequences to a complete mitochondrial genome sequence of a Chinese mitten crab (GenBank acc. KY041629) in Geneious v. 9.1.8 (Kearse et al., 2012). We chose the genetic locus for subsequent analyses for which data existed from both the native and introduced range, a fragment of the gene for the cytochrome c oxidase subunit one (COI).

For the phylogenetic reconstruction, we included COI sequences of all *Eriocheir* species and two outgroups. Most GenBank data consisted of haplotypes, not the actual sequences for each sampled individual. For the population genetic analyses, we reconstructed original haplotype frequencies by replicating haplotype sequences according to the data reported in the publications, attaching locality information to these sequences, and excluding sequences without sampling site information.

Phylogenetic reconstruction

The first step was to assert the species affinities of all sequences within a phylogenetic framework. For this, we used all sequences available in GenBank and BOLD. We built a maximum likelihood tree with the PHYML (Guindon et al., 2010) plugin in Geneious v. 9.1.8 (Kearse et al., 2012) under the General Time Reversible substitution model, estimating the transition/transversion ratio, the proportion of invariable sites and the gamma distribution parameter. The number of substitution rate categories was set to four. Branch support was calculated from 100 bootstrap replicates. The goal of this phylogenetic reconstruction was not to understand interspecific evolutionary relationships, but to ensure the lineage affinities of the sequences given the recent taxonomic confusion (Chu et al., 2003; Tang et al., 2003; C. Wang et al., 2008; Xu et al., 2009), and similar morphology of the species (Naser

et al., 2012).

Population genetic analyses: The search for invasion sources

All population genetic analyses were conducted in R version 3.3.3 (R. C. Team et al., 2018). We constructed a parsimony network of all haplotypes for each species that was found in Europe or the United States with the function 'haplotype' in the R package 'haplotypes' (Aktas, 2015), highlighting native and introduced populations. We visualized the geographic haplotype distribution in the native and introduced range by adapting available scripts for haplotype networks. We then identified haplotypes found in both the native and introduced range and evaluated their distribution in the native range to identify possible sources for invasion.

For Chinese mitten crabs, the species with the most complex invasion history, we conducted further population genetic analyses to understand invasion patterns and get additional support for probable source populations. We compared haplotype and nucleotide diversity for all sampling sites, which we also refer to as populations. We wrote our own function to calculate haplotype diversity of each population based on the formula of Nei and Tajima, 1981, and used the function 'nuc.div' of the 'pegas' package (Paradis et al., 2004) to calculate nucleotide diversity (Nei, 1987). We assessed if diversity indices were significantly different between native and introduced populations using standard ANOVA. We calculated Tajima's D, which may indicate population size changes or selective sweeps, using the function 'tajima.test' of the 'pegas' package (Paradis et al., 2004). Significant deviations from zero were estimated based on a beta distribution (Tajima, 1989).

We calculated genetic differentiation between all population pairs as ϕ_{ST} and Jost's D with the functions 'pairwiseTest' of the package 'strataG' (Archer et al. 2016), and the function 'pairwise_D' of the 'mmod' package (Winter 2012), respectively. ϕ_{ST} is a derivative of the classical fixation index F_{ST}, developed for mitochondrial haplotype data (Excoffier et al., 1992). We estimated significant deviations from zero (no differentiation between population pairs) by comparison with an empirical distribution of ϕ_{ST} values based on 1000 permutations. Jost's D provides a more accurate measure of population differentiation when genetic diversity is high and the number of unique alleles per population is large (Jost, 2008). Significance was assessed by bootstrapping populations across 1000 replicates. Each measure of differentiation resulted in a large number of pairwise comparisons, which are difficult to interpret. Therefore, we visualized overall population similarity with Metric Multidimensional Scaling, using the function 'cmdscale', and with hierarchical cluster analysis, using the function 'hclust'. Both functions are part of the R package 'stats'. The analyses of population structure served on the one hand to assess how differ-

entiated the native populations were, and therefore to indicate how narrowly we might be able to pinpoint the sources of introduction. On the other hand, we identified the native populations that were most similar to introduced populations as candidate source populations for the invasions.

We tested whether the native populations were sufficiently diverse to confidently assign individuals from the invaded range using the R package 'assignPOP' (K.-Y. Chen et al., 2018), which employs supervised machine learning to evaluate the discriminatory power of genetic or non-genetic data by resampling cross-validation. This means that individuals from each population were randomly divided into training and test sets, and assignment tests were repeated through resampling training individuals 100 times. We used the R function 'assign.MC' to conduct Monte Carlo cross validation using 80% of individuals from each population as training data. The proportion of correctly assigned individuals provides an estimate of assignment accuracy, which we calculated with the function 'accuracy.MC'. In case of sufficiently high discriminatory power, we would assign individuals from the invaded range to native populations using the function 'assign.X'.

Amino acid substitutions: Indication of the potential for adaptation

We extracted the DNA sequence alignment for the haplotypes, which was generated during the construction of the parsimony network (function 'haplotype' of package 'haplotypes' and function 'write.dna' of package 'APE') (Aktas, 2015; Paradis et al., 2004) and imported it into Geneious v. 9.1.8 (Kearse et al., 2012). We mapped it to the complete mitochondrial sequence of a Chinese mitten crab from China (GenBank acc. KY041629), and translated the DNA sequences to their amino acid sequence. This translation allowed us to identify amino acid substitutions. We assessed the directionality of change from the most parsimonious ancestral haplotype using the parsimony network constructed earlier.

Results

Data sources

On September 25, 2018, we downloaded a total of 1 020 sequences for the genus *Eriocheir* from GenBank, including eleven complete mitochondrial genome sequences. From these, we extracted 106 COI sequences after aligning all sequences to the complete mitochondrial genome sequence of a Chinese mitten crab from China (Li et al., 2016). In addition, we included seven COI sequences of Chinese mitten crabs from BOLD that were not available in GenBank. Prior to analyses, we removed sequence

AF317334 of a Hepu mitten crab because it had unusually many substitutions towards one end of the sequence (a sign of poor sequence quality), and discarded sequence CBCC039-11 from BOLD because it was shorter than the other sequences, but otherwise of the same haplotype and sampling site as CBCC049-11. None of the remaining sequences translated any stop codons, which would have indicated sequencing errors or the presence of nuclear pseudogenes.

The final alignment for the phylogenetic reconstruction contained 141 COI sequences (553 bp long) deposited in GenBank and BOLD under the names of the following species: two sequences of *E. ogaswaraensis* (Xu et al., 2009), 45 sequences of Japanese mitten crabs (Tang et al., 2003; Xu et al., 2009), six sequences of Hepu mitten crabs (Chu et al., 2003; Naser et al., 2012; Tang et al., 2003; J. Wang et al., 2016), 82 sequences of Chinese mitten crabs (Chu et al., 2003; Czerniejewski et al., 2012; Hänfling et al., 2002; Li et al., 2016; Liu et al., 2015; Otto, 2012; Raupach et al., 2015; Sun et al., 2005; Tang et al., 2003; J. Wang et al., 2016; Xu et al., 2009) and two sequences of the outgroup species.

For the population genetic analyses, we removed the following sequences without sampling site information: AF105247, FJ455507, NC_011597 and FJ455505. The final COI dataset for population genetic analyses contained 455 sequences of Chinese mitten crabs from 45 populations belonging to 20 haplotypes and 38 COI sequences of Japanese mitten crabs from eight populations belonging to 14 haplotypes. We reconstructed the population haplotype frequencies only for these two species because we wanted to infer the invasion sources of European and US invasions. The population-specific sequence information for Japanese and Chinese mitten crabs are summarized in Suppl. material: Tables S10 and S11, respectively.

Phylogenetic reconstruction

Our phylogenetic reconstruction recovered five main lineages of the genus *Eriocheir*, in agreement with previous phylogenetic studies (Naser et al., 2012; Xu et al., 2009) (Suppl. material: Fig. S4). For legibility, we provide the phylogenetic reconstruction with haplotypes only, highlighting their occurrence in the invaded range (Fig. 14). Haplotypes from invasive individuals of *Eriocheir* belonged to Japanese mitten crabs (Europe), Chinese mitten crabs (Europe and North America) and Hepu mitten crabs (Western Asia). The invasion of Hepu mitten crabs into Iraq has been discussed in detail elsewhere (Naser et al., 2012), and we focus our analyses on Chinese and Japanese mitten crab lineages. The occurrence of Japanese mitten crabs outside of their native range has not been reported previously. The phylogenetic reconstruction recovered that five Euro-pean individuals identified as Chinese mitten crab actually grouped with Japanese mitten crabs (Fig. 14). One crab was collec-

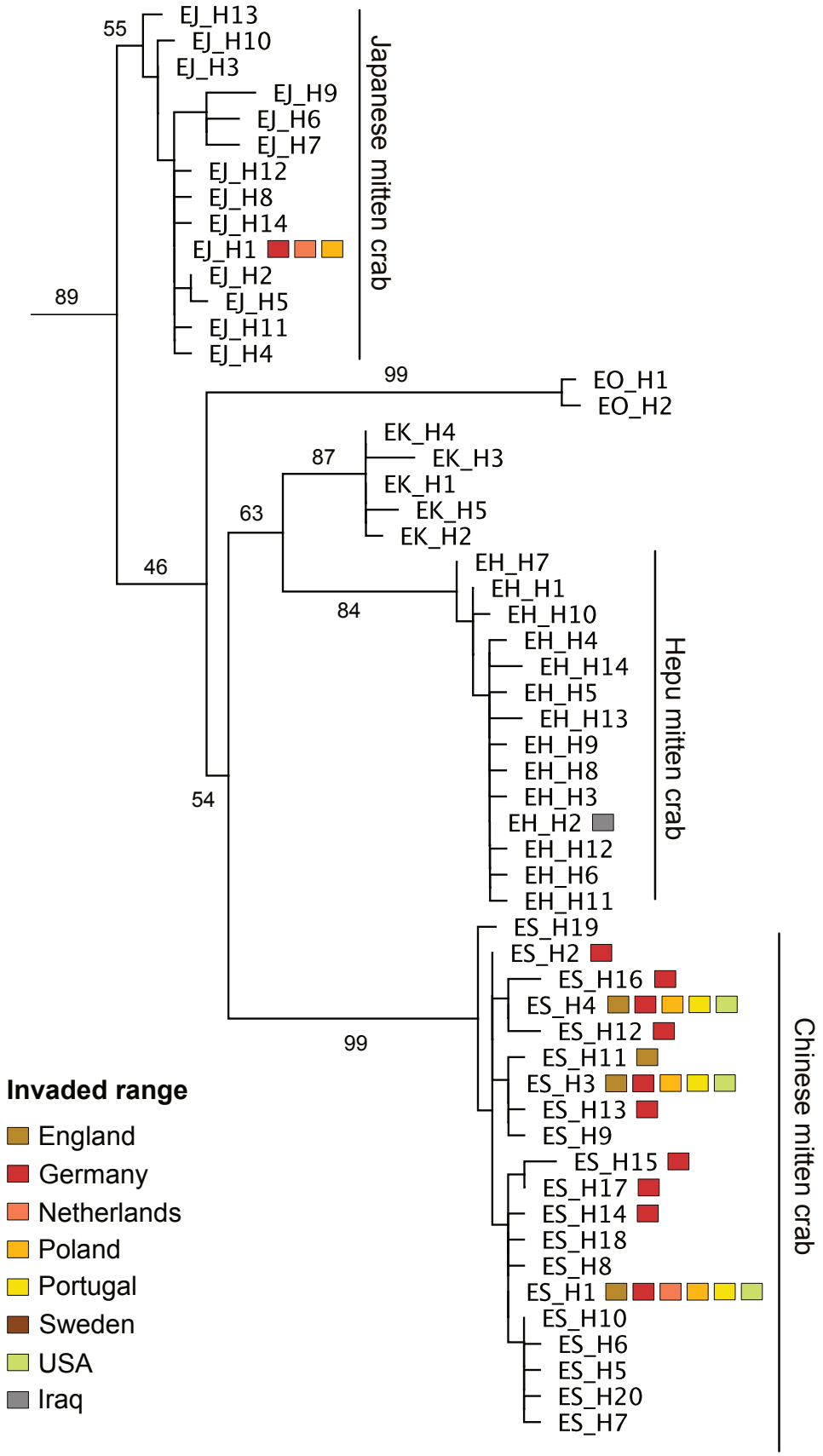

Figure 14: Maximum likelihood tree of mitten crab COI haplotypes. Haplotypes found in the invaded range are color-coded based on the country of collection. Numbers on branches represent bootstrap support. Branches without numbers have less than 50% bootstrap support. We omitted the outgroups from the figure to save space.

ted in Germany in 2009 (Raupach et al., 2015), one in Poland in 2015 by Dagmara Wojcik-Fudalewska (BOLD acc. OZ-IMP066-15), and three individuals caught in Holland and obtained from a seafood retailer were studied by Cristian Bernardi of the Universita degli Studi di Milano in 2011 (BOLD acc. CBCC038-11, CBCC039-11, CBCC040-11). Sequences of the Polish and Dutch individuals were only available in BOLD (http://www.boldsystems.org), not in NCBI GenBank, and have, to our knowledge, not been part of scientifically published studies. The origin of the latter was indicated in BOLD ambiguously as "Italy, Holland" without geographic coordinates but clarified in direct communication with C. Bernardi.

Population genetic analyses

We identified 14 haplotypes for Japanese mitten crabs, labelled H1 to H14 (Fig. 15B). The most common haplotype, H1, occurred in all populations but Shimonoseki, Japan. The invasive individuals found in Germany, Poland and Holland also had this haplotype, thus limiting our ability to assign a more detailed source population to invasive Japanese mitten crabs, and not meriting further population genetic analyses. We identified a total of 20 haplotypes for Chinese mitten crabs (Fig. 15A). Of these, nine haplotypes were found only in the native range, seven haplotypes only in Europe and four haplotypes in both native and introduced ranges. The geographic distribution maps visualize that haplotypes found only in the native region (blue colors) are common in central and northern Asia (Fig. 16A). Most of Europe is dominated by three of the four haplotypes shared between the native and introduced region (H1, H2, H4), which are more or less common in the native range: H1 was widespread in both the native and introduced range, thus providing little detail about the source of invasion (Fig. 16). H2 has a widespread distribution in its native range, found in two northern locations, Dalian City and Wuhu, and two central locations, Liaohe and an unspecified part of the Yangtze River (Fig. 16A, Suppl. material: Fig. S5). In the introduced range, H2 was found in two individuals only, one sampled in the Weser river near Oldenburg and the other in the Elbe river in Brandenburg, suggestive of its overall low frequency in the introduced range (Fig. 16D, Suppl. material: Fig. S5). H3 was reported from several central Chinese locations (Feiyunjiang, Hangzhou, Nantong, Yancheng, and Xhenjiang), and was widespread in Europe (Fig. 16, Suppl. material: Fig. S6). H4 was also widespread in Europe but reported in the native range only from Feiyunjiang (Fig. 16, Suppl. material: Fig. S7). In summary, three widespread invasive haplotypes were found in Feiyunjiang, making it a plausible main source for the invasion.

Several Northern German populations are genetically distinct: Aukrug, Eckernfoerde, Eider, Finkenwerder, Flemhude, Schlei, and Soholmer Au (marked with an

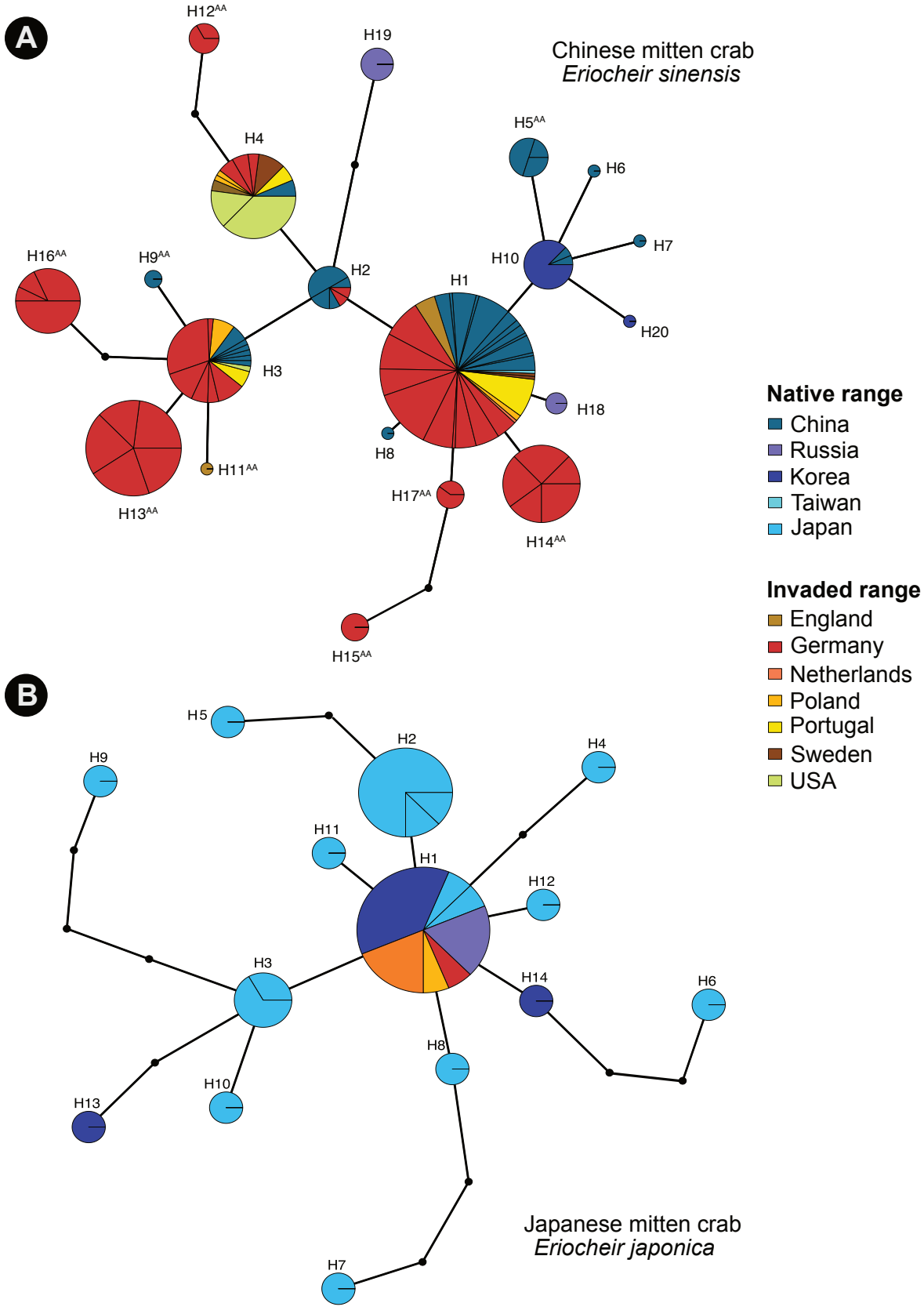

Figure 15: Parsimony networks for Chinese and Japanese mitten crabs. Each circle represents a haplotype, and the size of the circle is proportional to the abundance of this haplotype. **(A)** Chinese mitten crab: The smallest circle (e.g. H8) contains a single sequence, while the largest circle (H1) contains 161 sequences. **(B)** Japanese mitten crab: The smallest circle (e.g. H4) contains a single sequence, while the largest circle (H1) contains 18 sequences. The colors represent sampling sites: blue colors are native sites, yellow-orange-red colors are European sites and green colors are US sites.

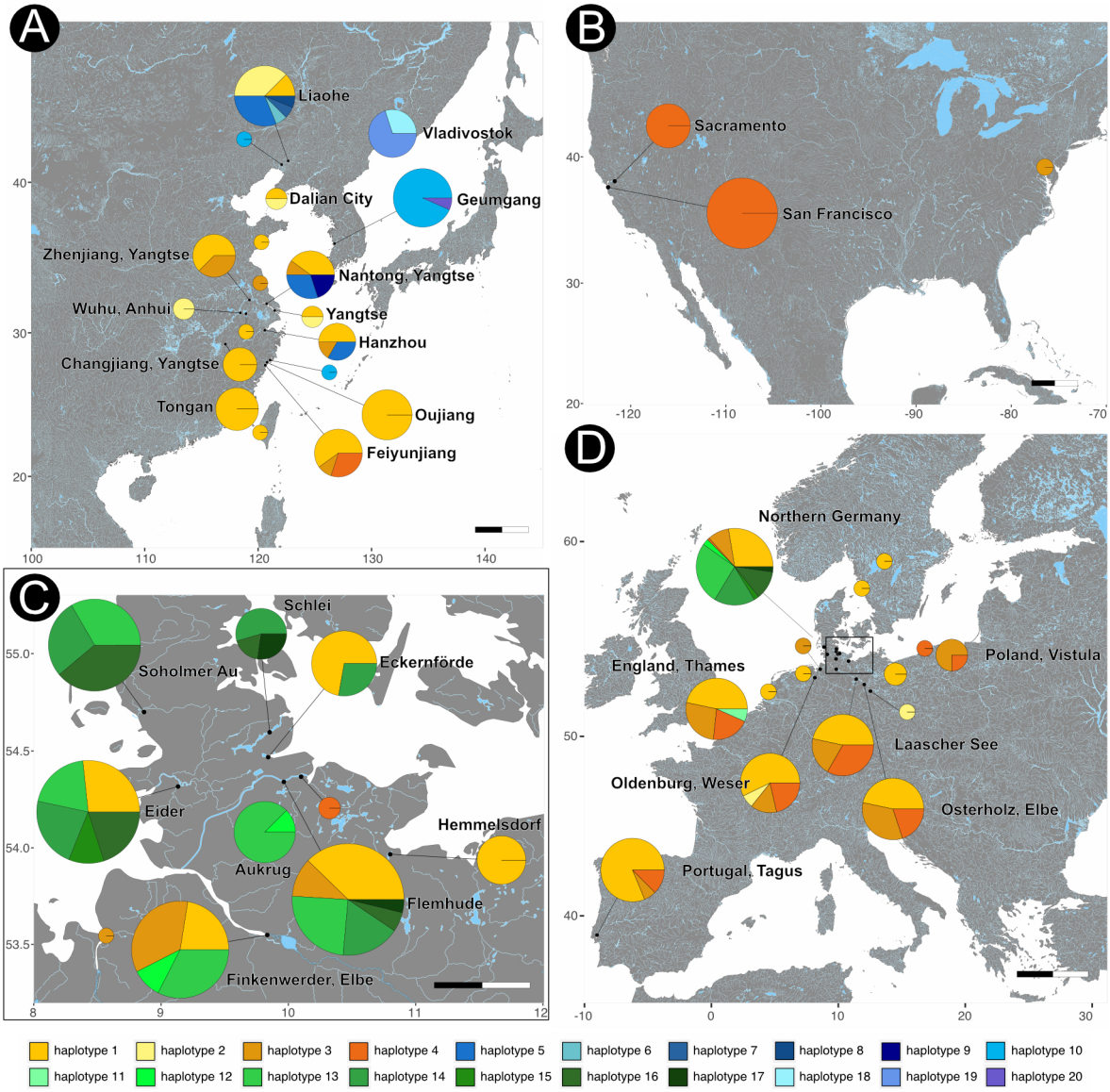

Figure 16: Geographic distribution of COI haplotype frequencies of Chinese mitten crabs. The distribution is shown in the native range **(A)**, the United States **(B)**, Northern Germany **(C)** and Europe in general **(D)**. Haplotypes only found in the native range are colored in blue tones, haplotypes only found in the introduced range are colored in green tones, and haplotypes found in both the native and introduced range are colored in yellow and orange tones. The smallest pie chart in each graphic represents a single individual. All populations with five or more sampled individuals are named. Additionally, in a we also point out the populations of Dalian City, Wuhu and Yangtze, as they contain the otherwise rare but invasive haplotype H2. Scale bars: 250 km **(A), (B), (D)**. 25 km **(C)**.

asterisk in Fig. 16C and D). These populations contained most of the haplotypes not detected in the native range, which we colored in green (compare Fig. 16A and C). In contrast to the European populations, the documented diversity in the US populations is lower. The large, established populations of the West coast seem to consist of a single haplotype, H4 (Fig. 16B), while a single individual sampled from an unestablished population on the East coast of the United States has a different haplotype (H3).

Overall haplotype diversity for Chinese mitten crabs was 0.832, and ranged from 0 to 0.805 per population (Table 5). Overall nucleotide diversity was 0.00384, and ranged from 0 to 0.00475 (Table 5). Introduced populations did not have lower haplotype diversity than native populations (Df = 1, F-value = 0.46, p-value = 0.505), nor did the nucleotide diversity between native and introduced populations differ (Df = 1, F-value = 0.453, p-value = 0.508). Tajima's D ranged from -1.159 to 2.315 per population (Table 5), and was not significantly different from zero in all but one population (Soholmer Au, D = 2.315, p-value = 0.021). In some cases, this could be the result of low sample size, which reduces the power to detect deviations from the null expectation. A total of nine haplotypes were private. They were distributed among four native sites (Liaohe: H6, H7, H8; Nantong: H9; Vladivostok: H18, H19; Geumgang: H20) and two introduced sites (Thames: H11; Eider: H15). Estimates of population differentiation among native populations with five or more sampled individuals revealed significant population structure across the native range (Suppl. material: Table S12).

Jost's D ranged from 0 to 0.215 and ϕ_{ST} from 0 to 1 (Suppl. material: Table S12). The overall pattern of relative differentiation was very similar between the two measures (Suppl. material: Table S12). Vladivostok in Russia and Geumgang in South Korea were significantly differentiated from all other native populations, and from each other. Some populations were undifferentiated with either distance measure, and clustered closely: 1) Oujiang and Tongan, both monotypic for haplotype H1, and 2) Hangzhou, Nantong and Liaohe.

We used these pairwise genetic distances to identify which introduced populations were genetically similar to native populations, representing potential sources of the invasion. In general, populations dominated by the same haplotype cluster together. The two monotypic Chinese populations, Oujiang and Tongan, cluster together with the German populations from Hemmelsdorf, Tagus and Eckernfoerde (Fig. 17). A second large cluster consists of several non-native populations from Germany and England and the Chinese population from Feiyunjiang and Zhenjiang (Fig. 17). These populations are undifferentiated with regard to Jost's D, which ranged from 0 to 0.003, and ϕ_{ST}, which ranged from 0 to 0.035. The Northern German populations Aukrug, Eckernfoerde, Schlei and Soholmer Au are significantly

Table 5: Results of population genetic analyses of Chinese mitten crab (*Eriocheir sinensis*) populations from sampling sites with more than five sampled individuals.

Sampling site	n	h	Haplotype diversity	Nucleotide diversity	Tajima's D	D p-value
Native range						
Feiyunjiang, China	10	3	0.600	0.002	0.473	0.636
Hangzhou, China	6	3	0.733	0.003	−0.057	0.954
Liaohe, China	16	6	0.783	0.003	0.095	0.924
Nantong, Yangtze, China	10	4	0.778	0.004	1.032	0.302
Oujiang, China	11	1	0.000	0.000	NA	NA
Tongan, China	8	1	0.000	0.000	NA	NA
Zhenjiang, Yangtze, China	8	2	0.536	0.002	1.449	0.147
Vladivostok, Russia	10	2	0.467	0.003	1.229	0.219
Geumgang, China	15	2	0.133	0.000	−1.159	0.246
Invaded range						
Thames, England	15	4	0.714	0.003	0.666	0.505
Aukrug, Germany	16	2	0.233	0.002	−0.744	0.457
Eckernfoerde, Germany	18	2	0.425	0.001	0.870	0.385
Eider, Germany	45	5	0.805	0.005	1.925	0.054
Finkenwerder, Elbe	40	4	0.729	0.003	0.758	0.448
Flemhude, Germany	53	6	0.766	0.004	1.171	0.241
Hemmelsdorf, Germany	10	1	0.000	0.000	NA	NA
Laascher See, Germany	15	3	0.676	0.002	1.386	0.166
Oldenburg, Weser, Germany	14	4	0.648	0.002	0.683	0.495
Osterholz, Elbe, Germany	15	3	0.676	0.002	1.386	0.166
Schlei, Germany	11	3	0.655	0.004	0.821	0.412
Soholmer Au, Germany	36	3	0.679	0.005	2.315	0.021
Tagus, Portugal	16	3	0.342	0.001	−0.708	0.479
Sacramento, USA	7	1	0.000	0.000	NA	NA
San Francisco, USA	18	1	0.000	0.000	NA	NA

Abbreviations: n: number of available sequences/individuals sampled; h: number of haplotypes.

differentiated from each other and all other populations. In the MDS plot of the first two coordinate axes (Fig. 17A), the Schlei population appears to lie within the first large cluster, but it is differentiated well by the third axis (Suppl. material: Fig. S8). Similarly, the US populations are differentiated from all other populations. In the introduced range at large, Jost's D ranged from 0 to 0.234, and ϕ_{ST} from 0 to 1. Within Europe, Jost's D ranged from 0 to 0.234, and ϕ_{ST} from 0 to 0.691, making European populations much more differentiated than native populations. The Monte Carlo cross validation procedure revealed little power to discriminate between source populations with assignment tests. The assignment accuracy averaged across replicates was 0.032. Thus, we did not attempt to assign invasive individuals to any particular native population with this method.

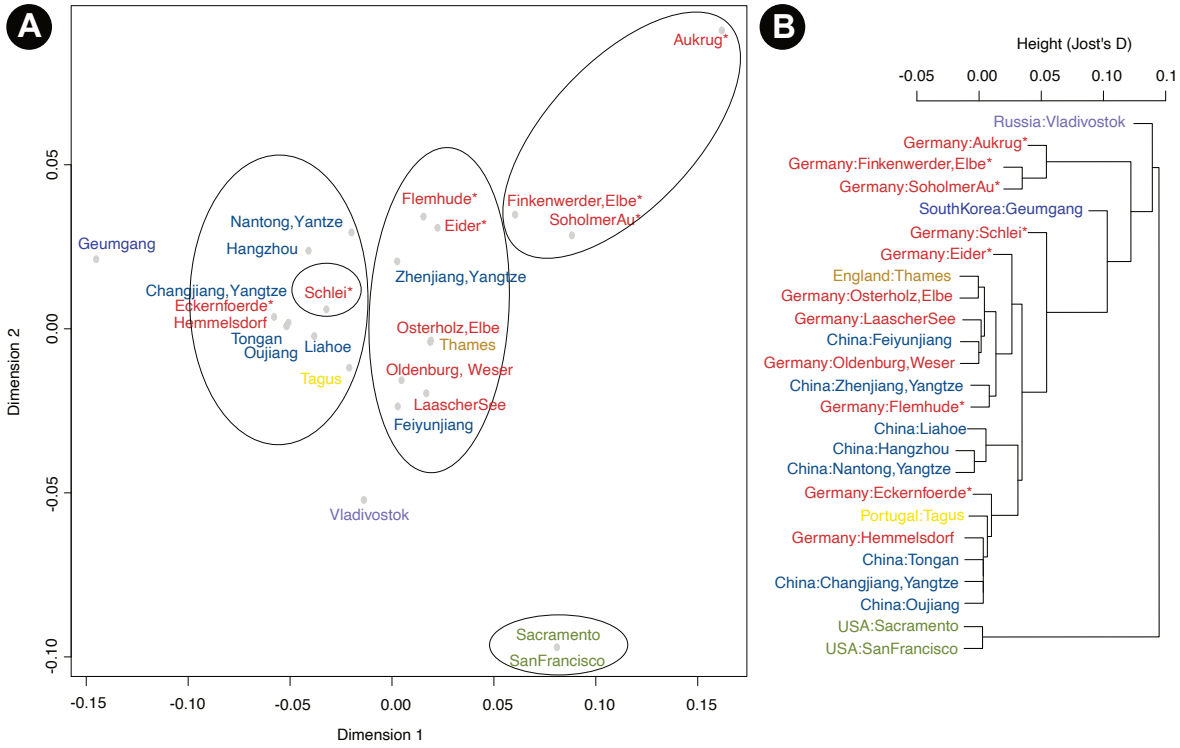

Figure 17: Visualization of genetic population similarity based on pairwise genetic differences calculated as Jost's D values. **(A)** multi-dimensional scaling plot. **(B)** hierarchical cluster analysis dendrogram. Asterisks denote Northern German populations that are dominated by haplotypes not found in the native range.

Amino acid substitutions

Amino acid substitutions took place in eight COI haplotypes: H5, H9, and H12 to H17 (Suppl. material: Fig. S9). Of these, the haplotypes H5 and H9 were only found in China, while H12 to H17 were the haplotypes only detected in Northern Germany. Based on the parsimony network, most substitutions occurred convergently. Only H15 evolved directly from H17. We can further infer the directionality of these substitutions from the haplotype network. It stands out that both proline and threonine evolved repeatedly in this small fragment of the COI gene.

Discussion

The Japanese mitten crab entered the European stage more than a decade ago

To our knowledge, we provide the first report of Japanese mitten crabs (*Eriocheir japonica*) outside their native range. Our phylogenetic reconstruction placed five sequences identified as Chinese mitten crabs clearly within the Japanese mitten crab lineage. The sequences were collected in Holland, Germany and Poland between 2009 and 2015. The German individual was collected inland in the Rhine river, and may not have necessarily migrated successfully to the North Sea for reproduction.

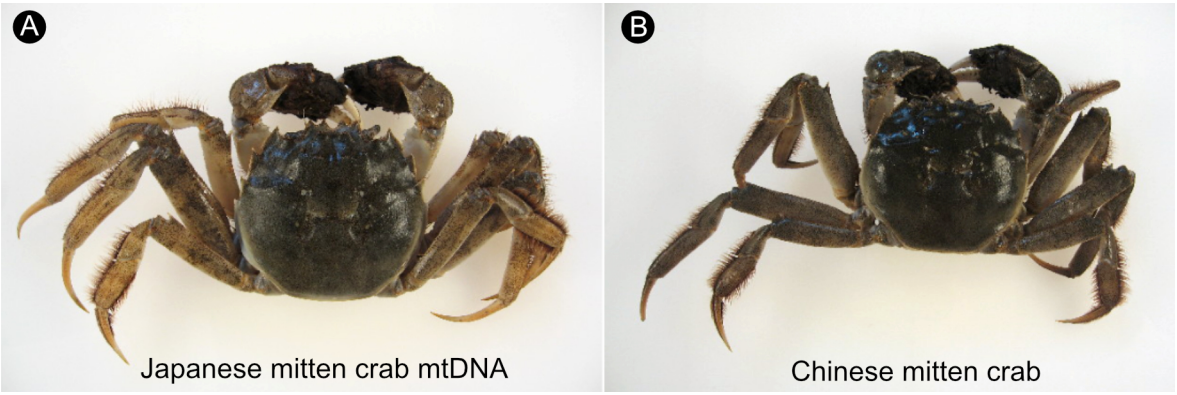

Figure 18: Mitten crabs collected in Holland in 2011. **(A)** Individual carrying mitochondrial DNA (mtDNA) of the Japanese mitten crab, morphologically identified as Chinese mitten crab, (BOLD accession: CBCC040-11, museum catalogue no: Universita degli Studi di Milano, Ispezione degli Alimenti di Origine Animale MALAC:00040). **(B)** Chinese mitten crab (BOLD accession: CBCC037-11, museum catalogue no: Universita degli Studi di Milano, Ispezione degli Alimenti di Origine Animale MALAC:00037). Photographs by Cristian Bernardi published under CC BY-NC 3.0 license.

The Dutch and Polish individuals were collected closer to the North and Baltic Sea, suggestive of an established, reproducing population of Japanese mitten crabs in Europe for the past ten years or more.

At first, it seems surprising that this invasion of Japanese mitten crabs has remained cryptic for at least a decade, but the morphological similarity between Chinese and Japanese mitten crabs did not make it obvious (Fig. 18) (Guo et al., 1997; Jensen and Armstrong, 2004; Naser et al., 2012). Moreover, all European sequences of Japanese mitten crabs were generated as part of sequencing efforts of between one and a few *Eriocheir* specimens (see BOLD records), diluting the meaning of the high genetic dissimilarity to other Chinese mitten crab sequences. Raupach et al., 2015, for example, actually discussed the high intraspecific genetic diversity of mitten crab sequences in their large barcoding study from German waters. They noted that the observed high genetic distances in their sample of Chinese mitten crabs were caused by a single specimen, which we assigned to the Japanese mitten crab based on its COI sequence. Morphological species identification placed this individual clearly as a Chinese mitten crab (Raupach et al., 2015). Similarly, according to the shape of the interocular carapace rim, the Dutch individuals would be identified as Chinese mitten crabs (Guo et al., 1997), but their COI sequences belong to Japanese mitten crabs. Interestingly, the Barcoding of Life Data System itself recognized that the five sequences in question clustered with Japanese mitten crabs, not with Chinese mitten crabs (http://www.boldsystems.org/index.php/Public_BarcodeCluster?clusteruri=BOLD:AAA8754). This discordance between morphology and mitochondrial sequence data may be due to the taxonomic confusion among *Eriocheir* species (Costa et al., 2007). The Monte Carlo cross validation procedure revealed little power to discriminate between source populations with assignment

tests. The assignment accuracy averaged across replicates was 0.032. Thus, we did not attempt to assign invasive individuals to any particular native population with this method. Cryptic morphology is a general problem in biological invasions that can only be resolved with molecular data. Bastrop and Blank, 2006, for example, used mitochondrial sequence data to show that in addition to the invasive polychaete *Marenzelleria neglecta*, two more species of the genus had invaded the Baltic Sea unnoticed. The invasive populations of the virile crayfish consist completely of a lineage not yet identified in the native North American range (Filipová et al., 2010). The cosmopolitan reed *Phragmites australis* represents an unusual case of cryptic invasion, where a non-native haplotype is currently replacing the native genetic diversity in North America (Saltonstall, 2002). The hypothesis of cryptic morphology is therefore clearly appealing and plausible. However, it is puzzling that all individuals identified as Japanese mitten crab at the sequence level were morphologically undoubtedly identified as Chinese mitten crabs. This discordance could hint at hybridization and subsequent introgression between Chinese and Japanese mitten crabs, resulting in morphological Chinese mitten crab hybrids carrying Japanese mitten crab mitochondrial genomes. This hybridization could have taken place either in the native or the invaded range. In such a case, pure Japanese mitten crabs do not necessarily have to form a stable population in Europe. Rather, their mitochondrial genomes would occur in some proportion of individuals with predominantly Chinese mitten crab genomes. Interspecific hybridization of another global invader has been confirmed for the shore crab genus *Carcinus* using a combination of mitochondrial sequence data and nuclear microsatellite data (Darling, 2011). To understand the current distribution of Japanese mitten crabs, possible hybrids between Japanese and Chinese mitten crabs or introgressed individuals in Europe, future systematic sampling, mitochondrial and nuclear sequencing of mitten crabs is highly warranted.

Significant genetic structure in the native and introduced range of Chinese mitten crabs

Much work has been conducted on the phylogeography of mitten crabs in their native range (Hänfling et al., 2002; Sui et al., 2009; C. Wang et al., 2008; Xu et al., 2009; D. Zhang et al., 2014; D. Zhang et al., 2012). Our re-analysis was therefore only aimed at assessing how useful the COI marker alone is to differentiate between populations, a prerequisite for identifying source populations with certainty (Geller et al., 2010), and to compare native and introduced diversity. We found that native populations are weakly but significantly differentiated, but this differentiation does not align with geography or river system, as noted previously (Sui et al., 2009). One reason

might be the exchange of crabs for commercial farming. Their extended planktonic larval period could also contribute to weak levels of genetic differentiation. This somewhat "chaotic" distribution of haplotypes makes it impossible to extrapolate the geographic distribution of genetic diversity, and precludes the assignment of broader regions as source regions. We can only discuss specific sampling sites as being more or less likely sources of introduction, as the genetic makeup of even the closest neighbor of any one site can be very different, e.g. in the case of Feiyunjiang and Oujiang. A more extensive sampling with regard to number of individuals and populations is certainly desirable to understand the patterns of diversity in the native range better. The fact that most populations were differentiated provides nonetheless a working baseline to assign source populations.

Most of the native populations had positive Tajima's D values, albeit not significantly different from zero, which is generally interpreted as populations being in mutation-drift equilibrium. It suggests that populations did not expand, shrink, or undergo recent selective sweeps at the mitochondrial genome. This pattern of genetic stability is anticipated for native populations. That we find the same pattern in most introduced populations is unexpected. We would expect to find negative Tajima's D values, indicative of recent bottlenecks. It seems unlikely that the introduced populations are already at equilibrium. Instead, an invasion of sufficient number of individuals that brought over a substantial amount of the native diversity could explain the observed pattern, either in a single or in multiple introduction events. In concordance with this idea, genetic diversity is not significantly lower in invasive populations, as would be expected when few individuals invade a new range.

The most distinct feature of the introduced range is the presence of seven haplotypes that have not been sampled in the native range. These haplotypes appear restricted to Northern Germany. Their distribution dominates the population structure in Europe, which divides populations with and without those unique alleles. We recovered more population structure than identified by Hänfling et al., 2002, and echo the findings of Otto, 2012, who generated and analyzed the Northern German COI data initially. She did not take the other known data into account, however, thus limiting her conclusions.

Plausible source populations of the Chinese mitten crab invasion

Hänfling et al., 2002 conducted the first search for source populations of the European and US invasion. Using COI sequence data, they identified five haplotypes that occurred in both China (three populations sampled) and Europe (five populations sampled). They did not find significant population structure in either the native or

introduced range, but observed a significant differentiation between those two. This was due to a haplotype common in all European and US populations, but absent in the native range. They used the presence of this haplotype as evidence for multiple introductions into Europe, and a secondary introduction of the United States from Europe. We identified this haplotype (H4) in one site in the native range, in Feiyunjiang. The other two haplotypes from Feiyunjiang (H1 and H3) are also common in Europe and the US, making this location the most plausible source of the invasion of those sampled so far. Feiyunjiang is located between the large ports of Shanghai and Xiamen (compare Figs. 13 – 17), which are suitable donor locations, each of the many departing commercial vessels from their ports acting as potential invasion vectors. The results of the analysis of pairwise population differentiation are concordant with these findings. Feiyunjiang clusters with several of the introduced populations, and is not significantly differentiated from them. The last haplotype found in both native and introduced range is H2. It was not detected in Feiyunjiang, but was present in Dalian City, Wuhu, part of the Yangtze River and Liaohe. Any of these locations could therefore be the source population of a second independent invasion into Europe. Unfortunately, the first three sites are only represented by two individuals each, making detailed comparisons of haplotype compositions between these native and invaded sites impossible. Alternatively to a second independent introduction event from a different location, this haplotype might occur in low frequencies in Feiyunjiang, but was not recovered there due to small sample sizes. In this case, the colonization of Europe by all the above-mentioned haplotypes could have been due to a single successful invasion event. Given the low frequency of the haplotype H2 in the invaded range, this is clearly a possibility. In general, the large native range is under-sampled with regard to the number of individuals and number of populations (Geller et al., 2010; Muirhead et al., 2008). This becomes especially important given the weak and chaotic population structure across the native range, which limits our power to predict the region of origin. It is, however, highly unlikely that the northern range of Chinese mitten crabs, e.g. Russia and South Korea, where we recovered only haplotypes absent from Europe and the United States, was the source of the invasion.

The US populations of Chinese mitten crabs have been speculated to be secondarily introduced from Europe (Hänfling et al., 2002). Based on our analyses, this remains a possibility, as the West coast populations are of a single haplotype, which was only found in Feiyunjiang in the native range, but is widespread in Europe. However, whatever led to the successful invasion of Europe from Feiyunjiang (or another native population with similar haplotype composition) might also have led to the successful invasion of the United States. The low genetic diversity of these populations certainly argues for the invasion of few individuals. In contrast, the

genetic diversity of European invaders is indicative of the invasion of several individuals. A single sequence available for a Chinese mitten crab from the East coast of the United States from an unestablished population (Benson and Fuller, 2019) is genetically distinct from the monotypic West coast population, advocating for an independent invasion of the East coast. The East coast haplotype (H3) is present in Europe, but is also relatively wide-spread in China, making both a secondary invasion from Europe, or a direct invasion from Asia, equally likely. A secondary invasion from the West coast of the United States is, however, highly unlikely. The analyses of genetic distances between populations echo on the one hand some of the results obtained by the comparison of haplotype identity between native and invaded ranges, and highlight, on the other hand, some of the difficulties associated with population genetic analyses of non-equilibrium scenarios pervasive during invasions. We found two clusters of mixed native and introduced populations: the first cluster contained populations from across Europe and Feiyunjiang. In line with the results of haplotype identity, Feiyunjiang is therefore a plausible source population. The second cluster contains populations monotypic for the most widespread haplotype. In this case, the native populations of Tongan and Oujiang have the same genetic makeup as the introduced populations of Hemmelsdorf, Eckernfoerde and Tagus, but this similarity may well be due to small populations and strong drift in the introduced populations, which could have eradicated much of the genetic diversity. Thus the second cluster of native and introduced populations cannot be interpreted as a separate introduction.

The distribution and origin of the uniquely Northern German haplotypes

The restricted distribution of the haplotypes H12 to H17 in northern Germany could either reflect a snapshot taken during an ongoing expansion or ecological restrictions. The most recent samples included in our analyses are those Northern German samples with unique haplotypes collected between 2008 and 2010 (Otto, 2012). No sites outside of Northern Germany were sampled, which means these haplotypes may have already spread throughout the remaining European range. One indication of a recent origin or arrival of these haplotypes can be gained via a comparison with other studies that included sites close by. Hänfling et al., 2002 included a site in the Elbe river near Osterholz, from which they collected 15 crabs between 1999 and 2000 (see suppl. material: table S10). This site is downstream from the Finkenwerder site sampled by Otto, 2012. None of the crabs collected in the Elbe between 1999 and 2000 had any of these uniquely Northern German haplotypes that were common between 2008 and 2010. Herborg et al., 2007 analyzed six microsatellite markers for six European populations, including the same Osterholz site. Otto, 2012

also analyzed her samples with microsatellite markers, including four of the markers analyzed by Herborg et al., 2007. The raw data are not available (as is so often the case for microsatellite data), but at the four common microsatellite loci, the Finkenwerder samples from 2008 – 2010 show higher allelic richness (A = 9.2 – 10.2) than the Osterholz samples collected ten years earlier (A = 4.3 – 9.1) across all four loci. While this is suggestive of a recent addition of genetic diversity, it does not preclude a restricted distribution of those haplotypes either, as Chinese mitten crabs commonly show genetic structure within the same river systems (Herborg et al., 2007; Sui et al., 2009). A broad sampling of current mitten crab genetic diversity in the invaded range would clarify how widely distributed those haplotypes really are.

The origin of the haplotypes only found in Northern Germany remains mysterious. If we interpret the absence of those haplotypes in the Osterholz samples, and the presence of two of these haplotypes in the Finkenwerder samples ten years later as the recent and simultaneous addition of these haplotypes to Europe, the most plausible scenario is a cryptic invasion from an unsampled native site. The source of such a cryptic invasion might be located in the northern range of Chinese mitten crabs. Overall, the number of analyzed native populations was rather small given the large range of Chinese mitten crabs (Fig. 16A). The available data did not include some large ports, such as Tianjin, Nampo, Daesan and Hungnam, all located in the northern range of Chinese mitten crabs (Fig. 13), which could be suitable donor areas. Based on the known distribution of Chinese mitten crabs, the very large ports around Hong Kong can be excluded as the invasion source because Hepu mitten crabs, not Chinese mitten crabs, occur in southern China (C. Wang et al., 2008; Xu et al., 2009) (Fig. 13).

Under a scenario of multiple invasions, the amino acid substitutions we found in all of the uniquely Northern German haplotypes evolved in the native range, and were introduced during the cryptic invasion. Whether these haplotypes confer indeed a selective advantage cannot be answered with certainty. They may carry, in fact, neutral or slightly deleterious mutations but have been swept to high frequencies during a recent strong selection event at a linked region of the genome (J. M. Smith and Haigh, 1974). The contemporaneous discovery of a novel physiology and behavior in Northern German mitten crabs, which allows them to complete the larval cycle in the brackish Baltic Sea Otto, 2012, may not be a coincidence. The expected range expansion caused by this novel ecology has already been documented by the recent and widespread occurrence of ovigerous females in the Eastern Baltic Sea (Ojaveer et al., 2007). Similarly, the occurrence of mitten crabs throughout much of the freshwater system of Sweden was hard to explain, invoking long-distance migration of crabs from their North Sea spawning grounds (Drotz et al., 2010). We suspect that these crabs belong to the same physiological type as the crabs investig-

ated by Otto, 2012, and are able to complete their larval cycle in the Baltic Sea.

Alternative hypotheses to a novel introduction can explain the origin of these uniquely Northern German haplotypes. In our opinion, the second most likely explanation is that the unique haplotypes evolved in the introduced range. Given that all of these haplotypes had one to three AAS, these haplotypes might have evolved rapidly in the introduced range in response to novel ecological conditions. Moreover, all of these uniquely Northern German haplotypes are closest related to a haplotype that was already present in Northern Europe (Fig. 15A). An argument against this hypothesis is that we would have expected to find some temporal sequence of haplotype evolution, with at least a few of the haplotypes occurring in earlier samples. Another hypothesis is that these haplotypes have been in Europe since the initial introduction, but either only recently increased in abundance, or were always restricted to Northern Germany, which had not been sampled before 2008. While genetic structure among sites or sampling years of the same river system exists (Herborg et al., 2007; Sui et al., 2009), crabs have to migrate along rivers to get to their marine mating and breeding grounds. Thus some mixing of haplotypes should occur along the river. Given the small sample sizes of older sites, a recent expansion of these haplotypes from standing variation is clearly possible. It is not obvious, however, why these haplotypes would have remained at very low frequencies for about 100 years, since their introduction.

Lastly, we cannot ignore the fact that all sequences with uniquely Northern German haplotypes were collected by Otto, 2012. If she had fallen prey to sequencing errors, we might expect randomly changed bases throughout each sequence, leading to many haplotypes present only once, and/or to the presence of stop codons. The fact that she sequenced many individuals with the same haplotype, and these translate to functional amino-acid sequences, makes sequencing error an unlikely source of these haplotypes. Furthermore, DNA fragments with uncertain base calls were sequenced in both directions (Otto, 2012), which should remove possible sequencing artifacts caused by faulty sequencing chemistry (Wares, pers. comm.).

At this point, we cannot determine if the high and unique haplotype diversity of Northern Germany is due to novel, potentially adaptive mutations that occurred after introduction, or due to multiple invasions. To clarify the origin of the unique haplotypes, we propose three approaches. Firstly, a more extensive sampling of the native range should identify if these haplotypes are present in the native range. Such sampling has already taken place (Sun et al., 2005; Tang et al., 2003), but we were unable to incorporate these data into our study because they used genetic markers not yet applied to the introduced populations. Thus we propose that future genotyping efforts of invasive specimens should include these genetic markers as well. Secondly, population genetic analyses of invasive mitten crabs from museum col-

lections could identify the temporal pattern of haplotype appearance. A sudden appearance of all unique haplotypes during the invasion history would hint at a new invasion event, while a stepwise appearance of novel haplotypes would be consistent with their evolution within the introduced range. Such pattern would, however, also be consistent with multiple additional invasion events, each introducing one or a few novel haplotypes. Lastly, population genomic analyses of native and invasive mitten crabs might reveal if the potentially adaptive haplotypes arose within invasive populations, in which case most of the genome of introduced Chinese mitten crabs should be undifferentiated and only small regions be highly differentiated. In contrast, a second introduction should show a more or less even differentiation across the genome. These efforts are aided by the recent publication of the nuclear genome of Chinese mitten crabs (Song et al., 2016) as well as the complete mitogenomes of several mitten crab species (Li et al., 2016; Liu et al., 2015).

Conclusion

This study uncovered complex population genetic pattern of invasive mitten crabs. Some of our findings are unambiguous, such as the presence of the mitochondrial genome of a second mitten crab species, the Japanese mitten crab, in Europe, suggesting either a cryptic invasion of this species or previous hybridization between Chinese and Japanese mitten crabs. This new European addition was only revealed by our data synthesis, which included barcoding data collected from various entities of a few individuals. The genetic diversity within European populations of Chinese mitten crabs remains puzzling, including the presence of several amino acid substitutions in haplotypes found only in Northern Germany. Taken together with the contemporaneous occurrence of a novel physiology and behavior in the same populations, it is possible that carriers of this haplotype have an adaptive advantage. Given the negative impacts of mitten crabs as an invasive species, we can only urge to monitor these invasive populations closely, using genetic tools such as the commonly used barcoding locus COI (Darling and Blum, 2007). Simultaneously, genomic and historical data could greatly enhance our understanding of the invasion process. We show that mitten crabs in Europe are melting pots of genetic diversity (Geller et al., 2010), making them prime targets to study cryptic invasions and possibly also rapid adaptations.

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Discussion

This thesis highlights the importance of museum collections. Natural history collections store selective moments of the past in the form of individual organisms that are linked to a specific date and place in time. Thus, the pure acquisition and interpretation of collection data made it possible to reconstruct the historic distribution changes of *Ostrea edulis* and *Crepidula fornicata*.

As the European flat oyster was an ecologically and still is an economically important species in Europe, many studies already exist on the decline of this native mollusc and its possible causes (e.g. Gercken and Schmidt, 2014; Möbius, 1877; Yonge, 1960). However, these studies have either emerged in the late 19th century when the oyster was still common or when it already was extinct in the North Sea in the 1930s. In consequence, many reasons have been discussed why *O. edulis* went extinct in the North Sea, but all these discussions are based on speculations. To address just one hypothesis among many, the introduced species *C. fornicata* was accused to compete with the native oyster for food and subsequently displacing *O. edulis*. Therefore, using the museum collection individuals as a window into the past, this is the first study that investigated this hypothesis on actual samples collected at the time in question. By combining several small and large collections, a huge database was generated that covers several European coastlines. Chapter 1 of this thesis clearly depicted the distribution changes of live oysters and empty shells, illustrating the extinction event in the North Sea. In parallel it could be shown that the population expansion of the introduced *C. fornicata* increased not until ten years after the last live oyster was collected. This study makes it clear that *C. fornicata* is not involved in the population decline of *O. edulis* in the North Sea.

Contrary to the expectations that introduced species become invasive and are mostly seen as a hazard to the native species and ecosystems, it was now proven that *C. fornicata* did not have a negative impact on the European flat oyster, but is more an enrichment of biodiversity. Since *C. fornicata* is a shallow water filter feeder as well as *O. edulis*, the snail may as well have taken over the ecological function of the European flat oyster in the ecosystems of the North Sea.

Studies based on museum collection material therefore provide the possibility to

reliably answer research questions on occasions in the past. Only when the unresolved questions of the past have been clarified, reasonable resettlement and conservation measures concerning *O. edulis* can be initiated. Many conservation management studies analysed the present-day populations of *O. edulis* regarding their distribution, phylogeography and differentiation pattern (Beck et al., 2011; Diaz-Almela et al., 2004; Lallias et al., 2010; Rodriguez-Perez et al., 2019; Vera et al., 2016). The investigations revealed a relatively homogeneous continuum of haplotypic frequencies in small and isolated populations (Diaz-Almela et al., 2004; Lallias et al., 2010). One explanation for this differentiation pattern would be the restoration strategies of the past, which included translocations of oyster spat to depleted oyster beds (Bromley et al., 2016). Oyster spat was translocated in high numbers repeatedly across Europe for about a century (Bromley et al., 2016). Additionally, latest studies about the consequences of translocating oysters states that populations are adapted to local environments, which also have an impact on reproduction and recruitment (Andrews, 1980; Bromley et al., 2016; McGinnity et al., 2009). This statement could be very informative for conservation managements and their restoration program. In order to resettle oysters successfully to extinct ranges, the historic phylogeography pattern of *O. edulis* will be most helpful to select the appropriate source population. In chapter 2 of the thesis, DNA of dry shells of the European flat oyster was extracted with ancient DNA methods to reconstruct the genetic differentiation pattern in the late 19th century. To our knowledge, this is the first study that successfully reconstructed the historic phylogeography of a species in its extinct range based on large scale museum collection material. The results show that the Wadden Sea was once inhabited by an autochthonous population of *O. edulis*, which so far has only been proven in this area and only from the late 19th century. Assuming that the populations were locally adapted with distinct tolerances and adaptations to their environment (Korringa, 1957; Loosanoff and Nomejko, 1951; Nelson, 1928), the result of this study would explain why the Wadden Sea has not been resettled by native oysters yet, after the autochthonous population went extinct. This finding of the so far unique haplogroup of *O. edulis* underlines the importance of museum collection material. Up to date, the extinction event of the European flat oyster was mainly explained by overfishing, strong winters and diseases (Gercken and Schmidt, 2014), but with the discovery of the lost haplogroup, the complete extinction event of the European flat oyster can now be analysed in a different light. Conclusively, extracting and interpreting DNA from museum collection material has helped before to track changes in genetic diversity through time, evaluate population genetic viability and identify specific candidate adaptive alleles in order to improve conservation management (Der Sarkissian et al., 2015).

Therefore, museum collections also serve as metagenomic archives of species as

was first proven by Doherty and Was, 2007. The analysis of mollusc shell DNA not only extracts endogenous DNA, but also microbial DNA (Der Sarkissian et al., 2017). In the course of the phylogeographical study, 34 oyster shells were sequenced, but did not contain any oyster DNA. Probably, these sequences may be consistent with the microbial content of oyster shells, or they may contain just contaminations resulting from the storage conditions in the museum collections. Presently, this microbial profiling research is not yet mature, because of many methodological problems and missing references, but this method will one day help to understand the interplay between molluscs and climatic changes, pollutants and emerging pathogens (Der Sarkissian et al., 2017). In the future, this method could even investigate if pathogens were involved in the extinction event of *O. edulis* in the North Sea.

Oyster shells enclose a variety of information both of the organism and its environment within the foliated calcite inner shell microstructure (Yonge, 1960). Environmental conditions such as temperature, food availability, salinity, pollution and pathogens can be analysed through the investigation of the structure and biogeochemical composition of the shell. Chapter 2 of this thesis covered only the reconstruction of the historical phylogeographical pattern of *O. edulis*, but in the future, biochemical and structural studies of the collection shells should be conducted to analyse the environmental conditions of that time. By combining both studies, it could be verified whether the historic distribution pattern of *O. edulis* correlates with adaptation to the environment. This information could also help to improve strategies for oyster restoration involving translocations.

Translocations, as in the case of the European flat oyster, are not always intentional, but are in many cases carried out accidentally by shipping traffic (Katsanevakis et al., 2013). The common limpet slipper was unintentionally introduced to Europe, where it spread rapidly. With the help of the collections, the exact path of the spread could be proven with place and date in the early 20th century. However, the case of *C. fornicata* illuminates also the disadvantages of the collections. The path of the spread could not be traced from the very beginning. Due to the literature, it is known that *C. fornicata* arrived in England in 1870, but the collections recorded the first individual not until 56 years later in the Netherlands. This discrepancy can be explained by several reasons: First, although this thesis included most museum collections in Northern Germany, it only added selected collections of three other countries that had already digitalized and published their archives. Since not all collections were embedded in this study, some records may have been missed. Second, especially older records before 1900 are often not labelled accurately. The labels usually give only very rough information about the sampling location, whereby the sampling dates are usually missing completely. Therefore, the collections may well contain early evidence of *C. fornicata*, but due to the missing data, the slipper

limpets cannot be classified in the time series. Third, since the common limpet slipper was generally regarded as a harmful species to oysters, it may as well have been not collected but annihilated right away. Thus, natural history collections are only as substantial and informative as the documentation and sampling events are.

The digitization of collections is a great responsibility for natural history museums, since without its availability online, the full potential of these collections cannot be achieved. Without making the collections public, the specimens and information they hold are almost only accessible to scientists of the institution (Page et al., 2015). With public data, however, researchers are able to access collections globally with regard to their own research question. Up to date, more than 1.5 billion collection entries of more than 54 000 data bases can be accessed online via the GBIF database, which is a powerful resource for future studies (https://www.gbif.org). Yet alone the impact that the combination of the collections of the most important European natural history museums had on the study of the European flat oyster and the common limpet slipper is also made clear in this thesis.

Nowadays, online public databases are commonly used to share many different information worldwide. The information is not only related to specimens in natural history collections, but also to DNA sequences (e.g. NCBI GenBank, BOLD). By using already published sequences, but putting the data into another context, new results can be generated. In the course of chapter 3, published DNA sequences were used to re-evaluate the genetic diversity of the invasive species *E. sinensis* between the native and the invasive range. Based on the genetic diversity shared between native and introduced range, a tributary of the Yangtze River, Feiyunjiang, appears to be a possible source population for the original introduction of Chinese mitten crabs to Europe. Moreover, the genetic diversity was comparatively high in Europe, because of seven unique haplotypes. This diversity could have two different reasons: First, this may represent a cryptic introduction from an unsampled native location. The native range has a rich river system and not all rivers have been sampled yet. Secondly, *E. sinensis* may have adapted rapidly to the invaded environment. The unique haplotypes are only found in Northern Germany, which is the invasive area where the crabs have been documented the longest. Since it is known that the Chinese mitten crab is now able to reproduce in the Baltic Sea, which it was not capable of before (Panning, 1952), these unique haplotypes could represent the adaptation to the new environment. Especially the fact that adult crabs do not die after the reproducing migration (Otto, 2012), supports the hypothesis that adaptation and haplotypes might be linked. The longevity of the Baltic Sea population differs also from the populations in the native range.

While evaluating the genetic diversity of the Chinese mitten crab, the presence of the Japanese mitten crab *Eriocheir japonica* in Northern Germany was verified for the

first time. The sequences were mistakenly identified as *E. sinensis*, because of the morphological similarity between both species. It is therefore conceivable that all mitten crabs collected and analysed in Europe have been identified by scientists as *E. sinensis*, as so far only this species has been detected outside the native range. As such, the first evidence of *E. japonica* in Germany highlights the importance of public databases. Only by combining the DNA sequences against the background of a specific research question, the introduction of the Japanese mitten crab to Europe was detected. In this context, it is necessary to re-examine the collections of mitten crabs in Europe taxonomically in order to correct further possible misidentifications, to study the morphological differentiation of both species and to explain the introduction history of *E. japonica* by means of historical collections.

Collections and databases have thus shown that they are a most valuable tool to reconstruct distributions of both native and introduced species and how those distributions have changed over time. Due to the huge number of specimens or their metadata that are assigned to a locality and date, the collections represent the only large-scale and verifiable data available. The conducted studies of this thesis have therefore emphasized the results of former studies (Page et al., 2015; Suarez and Tsutsui, 2004). Such studies of distribution shifts are becoming increasingly important against the background of climate change. Distribution and diversity shifts can have great impacts ecologically and economically. Climate change affects the distribution of biotic communities directly resulting in migration shifts, distributional changes or extinction events, which can have consequences for the surrounding environment (Flores et al., 2012; Schweiger et al., 2010). The importance of measuring biodiversity changes is becoming much more evident as the economic effects are increasing (Franceschi and Kahn, 2003; Gilbert, 2014). Especially, *E. sinensis* is causing large economical costs due to its burrowing behaviour. With the presence of the Japanese mitten crab, the economic impact may increase further.

Investigations of this thesis could not prove that the effects of climate change are involved in the extinction event of *O. edulis*. To investigate the connection between climate change and the extinction event of the European flat oyster, biochemical and structural analyses of the shells should be conducted in the future. The genetical and phylogeographical study suggests that the populations of *O. edulis* are adapted with distinct tolerances to their environment. Limited by historical barriers and natural dispersal ability, the populations were restricted to a certain geographical range. By translocating the oyster spat to other areas, the individuals could have lost their adaptive advantage over other populations, which might have led to a decreased competitiveness, reproduction and recruitment. Translocations in this size range in combination with improved fishing techniques likely led to the local extinction of *O. edulis*. Therefore, it is reasonably that the decline of *O. edulis* in the North

Sea was accelerated by the consequences of Industrialisation and improved fishing techniques.

The introduction of *C. fornicata*, *E. sinensis* and *E. japonica* is the consequence of Industrialisation and globalisation. Shipping traffic became much faster with the new technologies and the distances between the continents can be overcome very quickly today. Fouling species or larvae in ballast waters are able to survive the travelling time and are thus able to settle in new habitats. But it is possible that the climate change will influence the distribution range and favour the spread of these species to yet unsuitable areas in the future.

Finally, as proven by these studies, archives hold a lot of different information in diverse forms, but – in response to a specific question – they tell a special story.

Conclusion

The importance of museum collections has been clarified during the studies of this thesis. Based on collections, extinction events can be analysed from different angles. By reconstructing the distribution change of an extinct species, the sequence of places where the species no longer occurs over time can be proven. As in *O. edulis*, it could be shown that the shallow oyster beds went extinct first followed by the deeper beds in the Doggerbank in the North Sea. Furthermore, by assessing the collection material with modern techniques such as ancient DNA methods, the historical phylogeography of extinct species can be reconstructed, which helps to understand the process of extinction. This analysis revealed an extinct population of *O. edulis* that was autochthonous to the Wadden Sea. Moreover, based on collections, the impact as well as adaptation processes of neozoa can be reliably investigated, which was shown at the example of *C. fornicata* and *E. sinensis*. The 'oyster pest' *C. fornicata* was proven to be innocent in the process of extinction of *O. edulis* in the North Sea, whereas new haplotypes of *E. sinensis* found in Northern Germany suggest an adaptation to the new habitat within the past 100 years after introduction.

Error corrections

Coming and going – Historical distributions of the European oyster Ostrea edulis Linnaeus, 1758 and the introduced slipper limpet Crepidula fornicata Linnaeus, 1758 in the North Sea

During the implementation of the supplementary tables, I noticed a collection entry of *Ostrea edulis*, which was erroneously included in the table (https://doi.org/10.1371/journal.pone.0224249.s004):

Table 6: Specimen details of *Ostrea edulis*, which was removed from the supplementary table S2.

catalogue number	facility	sampling date	sampling location	latitude	longitude	status	publication
2446323	NHML	1917	North America, United States, Sussex	38,45211	−75, 04819	found dead	https://data.nhm.ac.uk/object/51bd5e24-0af0-4786-b8ca-18db0e780b24/1597795200000

I removed it from the supplementary table S2 of this thesis, because the table should contain only collections entries from individuals sampled in Europe and this specimen was sampled in North America. The deletion of this collection entry does not change the results of the publication in Chapter 1 (https://doi.org/10.1371/journal.pone.0224249), because the results were calculated using the geographical coordinates. Thus, the specimen is not included in any statistical calculations. The only change would be in the overview table 1, since one entry was deleted. The corrected table follows:

Table 7: Numbers of collected specimens and collection records of *Ostrea edulis* and *Crepidula fornicata* from cooperating museums and from public databases of the museums in London (GB), Leiden (Netherlands) and Paris (France). Corrected numbers are shown in red.

Museum/collections	**Museum acronym**	**Records of *O. edulis***	**Indiv. of *O. edulis***	**Records of *C. fornicata***	**Indiv. of *C. fornicata***
Senckenberg Natural History Collection, Dresden, Germany	SNSD	1	1	8	20
Senckenberg Natural History Museum, Frankfurt, Germany	SMF	2	175	5	8
Zoological Museum Greifswald, Germany	ZIMG	2	> 3	/	/
Centre of Natural History, Hamburg, Germany	ZMH	20	68	/	/
Zoological Museum, Kiel, Germany	ZMK	93	509	11	107
Naturalis Biodiversity Center, Leiden, Netherlands	NMNL	146	851	97	> 495
Natural History Museum, London, UK	NHML	2	5	10	70
Museum for Nature and Environment, Lübeck, Germany	MNUL	3	18	1	5
Zoological Collections of the University Rostock, Germany	ZSRO	14	> 85	/	/
German Oceanographic Museum, Stralsund, Germany	DMM	6	28	13	> 34
Muséum National d'Histoire Naturelle, Paris, France	MNHN	5	5	/	/

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Supplementary Material

Table S1: Details of *Ostrea edulis* of the 19th century, chronologically ordered.

catalogue number	facility	sampling date	sampling location	latitude	longitude	status	publication
Mo 5/1	ZMK	ca. 1820	Germany, Schleswig-Holstein, North Frisian Wadden Sea	55,05600889	8,41318	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 270	ZMK	1843	Germany, Schleswig-Holstein, Sylt, oyster beds	55,13202111	8,557795833	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 18/2	ZMK	Sep 1868	Germany, Schleswig-Holstein, North Sea, west coast	55,05600889	8,41318	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 81/3	ZMK	1869	England, Essex, North Sea coast, estuary of the river Blackwater	51,75774667	0,883626667	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 83/2	ZMK	1869	England, Essex, North Sea coast, estuary of the river Roach	51,61561194	0,866526944	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 84/2	ZMK	1869	England, Essex, North Sea coast, estuary of the river Colne	51,78621444	0,987052778	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 85/2	ZMK	1869	England, Southern Bight, estuary of the Thames	51,51899806	0,786895556	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 98/3	ZMK	Apr 1869	France, Nouvelle-Aquitaine, la Tremblade, estuary of the river Tremblade, "green oysters", bred in the claires	45,84494194	−1,167708889	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 99/3	ZMK	Apr 1869	France, Bretagne, la Trinité, Atlantic coast	47,57774889	−3,052350833	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 100/1	ZMK	Apr 1869	France, Bretagne, la Trinité, Atlantic coast	47,57774889	−3,052350833	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 101/1	ZMK	Apr 1869	France, Bretagne, la Trinité, Atlantic coast	47,57774889	−3,052350833	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 102/17	ZMK	Apr 1869	France, Nouvelle-Aquitaine, Bassin d'Arcachon, Atlantic coast	44,61520194	−1,205154722	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 97/8	ZMK	17.04.1869	France, Nouvelle-Aquitaine, Île de Ré	46,190695	−1,307336944	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 86/7	ZMK	May 1869	England, Kent, North Sea coast, Whitstable, estuary of the Thames	51,51899806	0,786895556	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 88/1	ZMK	May 1869	England, Kent, North Sea coast, Herne Bay, estuary of the Thames	51,51899806	0,786895556	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 90/1	ZMK	May 1869	England, Hampshire, Hayling island, near Portsmouth	50,78553583	−1,029968056	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 94/2	ZMK	May 1869	England, Cornwall, Saltash, estuary of the river Tamar	50,31631194	−4,186820833	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 106/11	ZMK	May 1869	France, Provence-Alpes-Côte d'Azur, Toulon, Mediterranean coast, artificial oyster bed "la Seyne"	43,11568306	5,887596944	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 87	ZMK	06.05.1869	England, Kent, North Sea coast, Herne Bay, estuary of the Thames	51,51899806	0,786895556	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 89/12	ZMK	06.05.1869	England, Kent, North Sea coast, Reculver, estuary of the Thames	51,51899806	0,786895556	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 82/3	ZMK	10.05.1869	England, Essex, North Sea coast, estuary of the river Crouch	51,61987611	0,959016944	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 91/10	ZMK	11.05.1869	England, Hampshire, Hayling island, near Portsmouth	50,78553583	−1,029968056	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 92/4	ZMK	11.05.1869	England, Hampshire, Hayling island, near Portsmouth	50,78553583	−1,029968056	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 202	ZMK	Jun 1869	Germany, Schleswig-Holstein, Sylt	55,05824306	8,416306944	found dead	http://zmk.sesam.senckenberg.de/page/index.htm

Mo 46/10	ZMK	Dez 1869	Denmark, Limfjord	57,106105	9,783402778	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 118/4	ZMK	Jul 1870	Italy, Venetia, lagoon of Venice	45,18385111	12,25680278	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 72/1	ZMK	14.08.1870s	Germany, Schleswig-Holstein, North Sea, west coast, Amrum	54,71221306	8,357382778	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 53/1	ZMK	25.08.1870	Germany, Schleswig-Holstein, North Sea, west coast, oyster bed on Sylt near List	55,05824306	8,416306944	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 39/1	ZMK	27.12.1870	Germany, Schleswig-Holstein, North Sea, west coast	55,05600889	8,41318	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 25/1	ZMK	24.03.1871	Germany, Schleswig-Holstein, North Sea, west coast	55,05600889	8,41318	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 57/1	ZMK	May 1871	Germany, Schleswig-Holstein, North Sea, west coast, east of Sylt, “Huntje” oyster bed	54,97209639	8,467025556	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 51/14	ZMK	Aug 1876	Germany, Schleswig-Holstein, North Sea, west coast, oyster beds of Sylt	55,05824306	8,416306944	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8/1	ZMK	13.08.1876	Germany, Schleswig-Holstein, North Sea, west coast	55,05600889	8,41318	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 66/1	ZMK	Apr 1877	Germany, Schleswig-Holstein, North Sea, west coast, oyster bed near Hörnum on Sylt	54,79363194	8,294999722	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 56/2	ZMK	24.05.1877	Denmark, North Sea, west coast, east of Rømø, “Tagholm” oyster bed	55,11664889	8,564944722	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 71/1	ZMK	22.05.1877	Germany, Schleswig-Holstein, North Sea, west coast, Amrum, “Rochel Pahlen” oyster bed	54,68637306	8,297633889	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 73/8	ZMK	22.05.1877	Germany, Schleswig-Holstein, North Sea, west coast, “Westen Amrum” oyster bed	54,68189	8,303901944	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 68/8	ZMK	22.05.1877 / 23.05.1877	Germany, Schleswig-Holstein, North Sea, west coast, oyster bed near Hörnum on Sylt	54,79363194	8,294999722	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 58/6	ZMK	23.05.1877	Germany, Schleswig-Holstein, North Sea, west coast, east of Sylt, “Huntje” oyster bed	54,97209639	8,467025556	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 69/1	ZMK	1878	Germany, Schleswig-Holstein, North Sea, west coast	55,05600889	8,41318	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 75/1	ZMK	1878	Germany, Schleswig-Holstein, North Sea, west coast, “Süden Gröde” oyster bed	54,62192806	8,722115	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 80/3	ZMK	Mar 1878	The Netherlands, Zeeland, Oosterschelde, market oysters	51,88487306	4,02728	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
/	ZIMG	1879	England	51,671691	0,951666	unknown	
28652	ZIMG	1879	Holland	52,695517	5,434088	found alive	
Mo 13/3	ZMK	Aug 1879	Germany, Schleswig-Holstein, North Sea, west coast	55,05600889	8,41318	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 76/7	ZMK	Jul 1881	Germany, Schleswig-Holstein, North Sea, west coast, sedimentation tank of Husum	54,47018306	9,032643889	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 77/6	ZMK	26.08.1881	Germany, Schleswig-Holstein, 8-10 geogr. Miles northwest of Helgoland	54,24630694	7,747978889	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 54/2	ZMK	25.08.1882	Germany, Schleswig-Holstein, North Sea, west coast, east of Sylt	55,053805	8,637555	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 62/4	ZMK	26.08.1882	Germany, Schleswig-Holstein, North Sea, west coast, east of Sylt, “Huntje” oyster bed	54,97209639	8,467025556	found alive	http://zmk.sesam.senckenberg.de/page/index.htm

Mo 200/1	ZMK	Oktober 1882	England, Essex, Red Crag, Walton-on-the-Naze	51,86234806	1,289754722	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 64/3	ZMK	Aug 1884	Germany, Schleswig-Holstein, North Sea, west coast, Sylt near Morsum Odde	54,85443	8,429012778	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 59/3	ZMK	22.08.1884	Germany, Schleswig-Holstein, North Sea, west coast, east of Sylt, "Huntje" oyster bed	54,97209639	8,467025556	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 60/1	ZMK	23.08.1884	Germany, Schleswig-Holstein, North Sea, west coast, east of Sylt, "Huntje" oyster bed	54,97209639	8,467025556	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 61/3	ZMK	23.08.1884	Germany, Schleswig-Holstein, North Sea, west coast, east of Sylt, "Huntje" oyster bed	54,97209639	8,467025556	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 55/2	ZMK	24.08.1884	Germany, Schleswig-Holstein, Sylt	55,05824306	8,416306944	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 70/4	ZMK	29.05.1885	Germany, Schleswig-Holstein, North Sea, west coast, "Wylus" oyster bed on Föhr	54,75695389	8,541017778	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 48/1	ZMK	30.05.1885	Germany, Schleswig-Holstein, North Sea, west coast, south of Sylt, "Tayde Mochels" oyster bed	54,95041417	8,465652222	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 49/2	ZMK	30.05.1885	Germany, Schleswig-Holstein, North Sea, west coast, south of Sylt, "Tayde Mochels" oyster bed	54,95041417	8,465652222	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 65/1	ZMK	Sep 1886	Germany, Schleswig-Holstein, North Sea, west coast	55,05600889	8,41318	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Biv 0495	ZSRO	Nov 1890	The Netherlands, North sea coast	53,24137444	7,197393889	found alive	http://zsro.sesam.senckenberg.de/page/index.htm

Table S2: Details of *Ostrea edulis* of the 20th century, chronologically ordered.

catalogue number	facility	sampling date	sampling location	latitude	longitude	status	publication
Mo 8942/1	ZMK	24.07.03	03. VII., St. 78	54,8	5,816666667	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
2679157	NHML	1904	England, Devon, off Plymouth	50,35487	−4, 15021	unknown	http://data.nhm.ac.uk/object/aada862e-e7de-460b-979f-fbeae7659c68
Mo 192	ZMK	1905	Germany, Nordrhein-Westfalen, Wesel, river Rhine bank	51,70490639	6,4565275	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8945/4	ZMK	04.03.05	05. III., St. 2	65,91666667	3,283333333	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8943/1	ZMK	19.03.05	05 III., St. 25	54.375	5,266666667	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8944/1	ZMK	20.03.05	05. III., St. 27	54,86666667	5,95	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8946/1	ZMK	20.03.05	05. III., St. 27	54,86666667	5,95	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 192	ZMK	1905	Rhine River, Wesel, Germany	51,70491	6,456527	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8189	ZMK	Feb 06	06. II. N Kurre, nach N14, „Hornsriff Außengrund"	56,21666667	7,35	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
ZMA. MOLL. 415132	NMNL	1914-1918	France, Bretagne, Island Jersey	49,249955	−2, 255262	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.415132
ZMA. MOLL. 108456	NMNL	1915	The Netherlands, Zeeland, Bruinisse	51,667427	4,095682	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108456
RMNH. MOL. 320021	NMNL	1915	North Sea	56,511018	3,515625	unknown	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.320021

ZMA. MOLL. 108561	NMNL	14.08.1918	The Netherlands, Zeeland, Yersche	51,519109	4,017162	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108561
ZMA. MOLL. 108514	NMNL	1920	The Netherlands, South Holland, Scheveningen	52,08238	4,233919	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108514
ZMA. MOLL. 108478	NMNL	1920	The Netherlands, Zeeland, Dreischor	51,717487	3,984037	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108478
ZMA. MOLL. 108463	NMNL	1920	The Netherlands, North Holland, Den Helder	52,915776	4,712989	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108463
ZMA. MOLL. 114469	NMNL	1920	The Netherlands, Zeeland, Anna Jacobapolder St. Filipsland	51,60603	4,1731	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114469
ZMA. MOLL. 108519	NMNL	1920	The Netherlands, Zeeland, Anna Jacoba polder; St.Filipsland	51,60603	4,1731	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108519
ZMA. MOLL. 108578	NMNL	1920	The Netherlands, North Holland, Zandvoort	52,40027	4,53871	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108578
ZMA. MOLL. 108511	NMNL	17.06.1920	The Netherlands, Zeeland, Philippine at Isabella lock	51,31314	3,731766	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108511
ZMA. MOLL. 108553	NMNL	05.09.1920	The Netherlands, Zeeland, Walcheren	51,534664	3,433184	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108553
ZMA. MOLL. 114453	NMNL	15.06.1922	The Netherlands, Zeeland, Domburg	51,550543	3,456949	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114453
ZMA. MOLL. 108465	NMNL	15.06.1922	The Netherlands, Zeeland, Domburg	51,550543	3,456949	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108465
ZMA. MOLL. 108534	NMNL	25.06.1922	The Netherlands, North Holland, Texel	53,185393	4,85608	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108534
ZMA. MOLL. 108464	NMNL	1923	The Netherlands, Noord Holland, Den Helder	52,915776	4,712989	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108464
ZMA. MOLL. 415110	NMNL	Apr 1924	France, Bretagne, Iles des Glénans	47,724685	−4, 03536	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.415110
ZMA. MOLL. 415118	NMNL	22.04.1924	France, Bretagne, Locmariaquer	47,583716	−2, 989735	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.415118
ZMA. MOLL. 108518	NMNL	08.06.1924	The Netherlands, Zeeland, Schouwen	51,688916	3,786669	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108518
ZMA. MOLL. 114435	NMNL	12.10.1924	The Netherlands, Zeeland, Domburg, beach	51,550543	3,456949	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114435
ZMA. MOLL. 112556	NMNL	04.01.1925	The Netherlands, Noord Holland, IJmuiden	52,440319	4,556404	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.112556
ZMA. MOLL. 112560	NMNL	Jul 1926	The Netherlands, Friesland, Terschelling	53,351995	5,151671	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.112560
ZMA. MOLL. 114445	NMNL	Aug 1926	The Netherlands, North Brabant, Bergen op Zoom	51,511459	4,220558	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114445
ZMA. MOLL. 108448	NMNL	Aug 1926	The Netherlands, Noord Brabant, Bergen op Zoom	51,511459	4,220558	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108448

ZMA. MOLL. 108575	NMNL	13.08.1926	The Netherlands, Zeeland, Yerseke	51,519109.	4,017162	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108575
ZMA. MOLL. 108537	NMNL	24.08.1926	The Netherlands, Noord Holland, Texel	53,185393	4,85608	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108537
ZMA. MOLL. 108529	NMNL	24.08.1926	The Netherlands, Friesland, Terschelling	53,351995	5,151671	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108529
ZMA. MOLL. 108510	NMNL	Sep 1926	The Netherlands, North Holland, Petten-Camperduin	52,770647	4,662477	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108510
ZMA. MOLL. 114432	NMNL	Mar 1927	The Netherlands, Zeeland, Vlissingen	51,439545	3,601125	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114432
ZMA. MOLL. 108488	NMNL	18.02.1928	The Netherlands, North Holland, IJmuiden	52,440319	4,556404	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108488
ZMA. MOLL. 108548	NMNL	Aug 1928	The Netherlands, Friesland, Vlieland	53,206814	4,846498	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108548
RMNH. MOL. 319330	NMNL	Aug/Sep 1928	France, Bretagne, close to Roscoff	48,718385	−4, 013649	unknown	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.319330
ZMA. MOLL. 108503	NMNL	03.03.1929	The Netherlands, Zeeland, Oostkapelle	51,572002	3,508551	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108503
ZMA. MOLL. 108505	NMNL	13.03.1929	The Netherlands, Zeeland, Oranjezon	51,591636	3,559676	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108505
RMNH. MOL. 319327	NMNL	May-Jun 1929	United Kingdom	49,957089	−5, 215758	unknown	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.319327
ZMA. MOLL. 114459	NMNL	03.09.1929	The Netherlands, Zeeland, Oostkapelle	51,572002	3,508551	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114459
ZMA. MOLL. 108502	NMNL	1930	The Netherlands, Zeeland, Oosterschelde	51,566338	3,948963	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108502
RMNH. MOL. 320011	NMNL	16.06.1930	Croatia, Itria, Rovinj	45,129388	13,6866	found dead	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.320011
ZMA. MOLL. 415121	NMNL	1931	France, Ajaccio, Corsica, Pino beach	42,923502	9,358264	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.415121
ZMA. MOLL. 108492	NMNL	Jul 1930	The Netherlands, Utrecht, Kolenvoet near Woudenberg	52,117376	5,479951	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108492
ZMA. MOLL. 108570	NMNL	Sep 1932	The Netherlands, Zeeland, Yerseke	51,519109	4,017162	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108570
ZMA. MOLL. 114461	NMNL	Sep 1932	The Netherlands, Zeeland, Yerseke	51,518893	4,017248	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114461
ZMA. MOLL. 108445	NMNL	1933	The Netherlands, North Holland, Bergen aan Zee	52,656122	4,626454	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108445
ZMA. MOLL. 108576	NMNL	1934	The Netherlands, Zeeland, Yerseke	51,519109	4,017162	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108576
ZMA. MOLL. 108559	NMNL	25.09.1934	The Netherlands, Zeeland, Yersche Bank	51,518893	4,017248	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108559

ZMA. MOLL. 112547	NMNL	03.11.1934	The Netherlands, North Holland, Bakkum aan Zee	52,552257	4,604536	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.112547
ZMA. MOLL. 108458	NMNL	16.03.1935	The Netherlands, North Holland, Camperduin	52,718506	4,638638	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108458
ZMA. MOLL. 108494	NMNL	24.04.1935	The Netherlands, Zeeland, Noord-Beveland	51,592897	3,641021	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108494
ZMA. MOLL. 108476	NMNL	27.04.1935	The Netherlands, Zeeland, Domburg	51,550543	3,456949	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108476
ZMA. MOLL. 415126	NMNL	22.08.1935	England, Kent, Dover, Stat. 819	51,111174	1,295893	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.415126
ZMA. MOLL. 415105	NMNL	27.08.1935	France, Hauts-de-France, near Calais, Stat. 626	50,962752	1,829504	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.415105
ZMA. MOLL. 108533	NMNL	03.09.1935	The Netherlands, NorthHolland, Texel	53,185393	4,85608	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108533
ZMA. MOLL. 415127	NMNL	03.09.1935	France, Pas-de-Calais, Stat. 636	51,014255	2,091008	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.415127
ZMA. MOLL. 108441	NMNL	1936	The Netherlands, Gelderland, Arnhem	52,076775	5,946108	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108441
ZMA. MOLL. 108483	NMNL	1936	The Netherlands, Zeeland, Grevelingen near Bruinisse	51,667427	4,095682	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108483
RMNH. MOL. 93762	NMNL	1936	The Netherlands, North Holland, Texel, Oosterend	53,076115	4,894017	unknown	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.93762
ZMA. MOLL. 114474	NMNL	Feb 1936	The Netherlands, Zeeland, Grevelingen near Bruinisse	51,667427	4,095682	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114474
ZMA. MOLL. 114473	NMNL	Feb 1936	The Netherlands, Zeeland, Grevelingen near Bruinisse	51,66748	4,095553	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114473
47056	ZMH	15.06.1936	Italy, Naples	40,79175889	14,16375417	found dead	
ZMA. MOLL. 112554	NMNL	18.08.1936	The Netherlands, Zeeland, Yerseke	51,519109	4,017162	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.112554
ZMA. MOLL. 108560	NMNL	1937	The Netherlands, Zeeland, Yersche Bank	51,518893	4,017248	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108560
ZMA. MOLL. 114457	NMNL	1937	The Netherlands, Zeeland, Yerseke	51,519109	4,017162	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114457
ZMA. MOLL. 108565	NMNL	1937	The Netherlands, Zeeland, Yerseke	51,519109	4,017162	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108565
ZMA. MOLL. 114470	NMNL	28.02.1937	The Netherlands, North Holland, Bloemendaal	52,405576	4,541908	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114470
ZMA. MOLL. 108513	NMNL	31.03.1937	The Netherlands, South Holland, Limburg	51,984166	4,08105	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108513
ZMA. MOLL. 108480	NMNL	15.08.1937	The Netherlands, Friesland, Island Griend	53,254115	5,24607	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108480
ZMA. MOLL. 108532	NMNL	22.09.1937	The Netherlands, North Holland, Texel	53,185393	4,85608	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108532

ZMA. MOLL. 415109	NMNL	1938	Norway, Rogaland, Stavanger	59,000049	5,679206	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.415109
ZMA. MOLL. 114421	NMNL	1938	The Netherlands, Zeeland, Grevelingen	51,770309	3,863317	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114421
ZMA. MOLL. 108497	NMNL	1938	The Netherlands, Zeeland, Oosterschelde	51,566338	3,948963	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108497
ZMA. MOLL. 108556	NMNL	1938	The Netherlands, Zeeland, Westkapelle	51,550218	3,456721	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108556
ZMA. MOLL. 108446	NMNL	1938	The Netherlands, North Holland, Bergen aan Zee	52,656122	4,626454	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108446
ZMA. MOLL. 108449	NMNL	1938	The Netherlands, North Brabant, Bergen op Zoom	51,511459	4,220558	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108449
ZMA. MOLL. 108482	NMNL	1938	The Netherlands, Zeeland, Grevelingen	51,770309	3,863317	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108482
ZMA. MOLL. 108481	NMNL	01.04.1938	The Netherlands, Friesland, Island Griend	53,254167	5,245856	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108481
ZMA. MOLL. 112668	NMNL	30.09.1938	The Netherlands, Friesland, Kornwerderzand	53,060371	5,281093	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.112668
ZMA. MOLL. 108544	NMNL	05.10.1938	The Netherlands, Zeeland, Tholen	51,53553	4,228594	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108544
ZMA. MOLL. 114447	NMNL	05.10.1938	The Netherlands, Zeeland, Tholen	51,53553	4,228594	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114447
ZMA. MOLL. 108454	NMNL	Nov 1938	The Netherlands, Zeeland	51,369866	3,365205	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108454
ZMA. MOLL. 415107	NMNL	1939	North Sea, Oestergronden	54,916335	4,049292	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.415107
ZMA. MOLL. 114442	NMNL	1939	The Netherlands, North Brabant, Bergen op Zoom	51,511459	4,220558	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114442
ZMA. MOLL. 108451	NMNL	28.02.1939	The Netherlands, Noord Holland, Bloemendaal	52,405576	4,542251	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108451
ZMA. MOLL. 114466	NMNL	May 1939	The Netherlands, Zeeland, Yerseke	51,519109	4,017162	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114466
ZMA. MOLL. 114462	NMNL	Sep 1939	The Netherlands, Zeeland, Yerseke	51,518893	4,017248	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114462
ZMA. MOLL. 108573	NMNL	Sep 1939	The Netherlands, Zeeland, Yerseke	51,518893	4,017248	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108573
ZMA. MOLL. 108437	NMNL	Oct 1939	The Netherlands, North Holland, Amsterdam	52,347221	4,913268	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108437
ZMA. MOLL. 108485	NMNL	Nov 1939	The Netherlands, Zeeland, Grevelingen	51,770309	3,863317	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108485
ZMA. MOLL. 108504	NMNL	1940	The Netherlands, Noord Holland, Buiten-IJ	52,379597	4,911991	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108504

ZMA. MOLL. 108526	NMNL	Sep 1941	The Netherlands, Zeeland, Stavenisse	51,604018	4,042769	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108526
ZMA. MOLL. 108567	NMNL	Sep 1941	The Netherlands, Zeeland, Yerseke	51,519109	4,017162	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108567
ZMA. MOLL. 114465	NMNL	Sep 1941	The Netherlands, Zeeland, Stavenisse	51,598793	4,018908	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114465
ZMA. MOLL. 108571	NMNL	Sep 1941	The Netherlands, Zeeland, Yerseke	51,518893	4,017248	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108571
ZMA. MOLL. 114463	NMNL	Sep 41	The Netherlands, Zeeland, Yerseke	51,519109	4,017162	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114463
ZMA. MOLL. 114449	NMNL	1942	The Netherlands, Zeeland, Westerschelde	51,454344	3,430329	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114449
ZMA. MOLL. 108555	NMNL	1942	The Netherlands, Zeeland, Westerschelde	51,454344	3,430329	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108555
Mo 96/4	ZMK	Apr 1943	France, Bretagne, Ptc. de Penmarch	47,84173583	−4, 3512725	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 63/15	ZMK	Aug/Sep 1948	Germany, Schleswig-Holstein, North Sea, west coast, Sylt near the Zoological institute in List	55,02077806	8,439468889	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
W 180	MNUL	03.10.1948	Germany, Schleswig-Holstein, Husum, dyke	54,46591333	8,911403889	unknown	
ZMA. MOLL. 114440	NMNL	21.03.1949	The Netherlands, Zeeland, Rammekens	51,458446	3,662481	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114440
ZMA. MOLL. 108536	NMNL	Jun 1949	The Netherlands, Noord Holland, Texel	53,185393	4,85608	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108536
W 181	MNUL	Jul 1949	Germany, Schleswig-Holstein, Amrum, Wittdün	54,62905	8,384582222	unknown	
ZMA. MOLL. 108527	NMNL	18.08.1949	The Netherlands, Zeeland, Terneuzen	51,342849	3,81423	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108527
ZMA. MOLL. 108528	NMNL	31.08.1949	The Netherlands, Zeeland, Terneuzen	51,342849	3,81423	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108528
ZMA. MOLL. 108490	NMNL	29.10.1949	The Netherlands, Noord Holland, IJpolder	52,468866	4,562873	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108490
ZMA. MOLL. 114438	NMNL	Jun 1950	The Netherlands, Zeeland, Kalkbranderij Den Briel	51,929318	4,144029	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114438
Mo 74/2	ZMK	Jul 1950	Germany, Schleswig-Holstein, North Sea, west coast, Amrum	4,71221306	8,357382778	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
ZMA. MOLL. 108516	NMNL	26.08.1950	The Netherlands, Zeeland, Schouwen	51,717382	3,686281	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108516
ZMA. MOLL. 108582	NMNL	26.08.1950	The Netherlands, Zeeland, Zierikzee	51,667267	3,876441	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108582
ZMA. MOLL. 108552	NMNL	Apr 1951	The Netherlands, Zeeland, Vlissingen	51,439545	3,601125	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108552
ZMA. MOLL. 114437	NMNL	1952	The Netherlands, Zeeland, near Terhofstede	51,338325	3,392655	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114437
27847	SNSD	1952	Nordsee	56,511018	3,515625	unknown	https://search.senckenberg.de/aquila-public-search/search

ZMA. MOLL. 108549	NMNL	14.03.1952	The Netherlands, Zeeland, Vlissingen	51,439545	3,601125	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108549
Mo 1477	ZMK	Sep 1952	France, Provence-Alpes-Côte d'Azur, St. Maries de la Mér, estuary of the river Rhône	43,45101861	4,391612778	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 1474	ZMK	Nov 1952	Germany, Schleswig-Holstein, Sylt, northern tip of the island	55,05824306	8,416306944	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
RMNH. MOL. 320051	NMNL	1953	The Netherlands, South Holland, Bolnes	51,900386	4,573285	unknown	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.320051
Mo 1472	ZMK	Jul 1954	Denmark, North Jutland County, Hulsig near Skagen, Kattegat	57,66597278	10,49468972	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 1473	ZMK	Jul 1954	Denmark, North Jutland County, Skagen, Skagerrak	57,73110194	10,52387222	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 1476	ZMK	Aug 1954	Denmark, Thyholm, Limfjord, Oddesund near Odby	56,58974167	8,529510278	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
ZMA. MOLL. 125427	NMNL	05.09.1954	The Netherlands, South Holland, Oostvoorne, beach	51,922791	4,038344	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.125427
ZMA. MOLL. 108487	NMNL	31.10.1954	The Netherlands, Noord Holland, IJmuiden	52,440319	4,556404	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108487
ZMA. MOLL. 114441	NMNL	1955	The Netherlands, Zeeland, Veere	51,566958	3,637784	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114441
ZMA. MOLL. 108512	NMNL	23.07.1955	The Netherlands, Zeeland, Domburg	51,559456	3,503211	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108512
ZMA. MOLL. 108577	NMNL	14.06.1958	The Netherlands, Zeeland, Zandplaat De Hooge Springer	51,403445	3,594906	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108577
W 183	MNUL	1960	England, Isle of Wight	50,76660528	−1,296110833	unknown	
RMNH. MOL. 95500	NMNL	Jul 1960	France, Bretagne, St. Lunaire	48,635271	−2,124324	found dead	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.95500
47067	ZMH	Jul 1960	Adriatic Sea	41,85509028	17,29028389	found dead	
ZMA. MOLL. 108434	NMNL	06.05.1961	The Netherlands, Zeeland, Aardenburg	51,259988	3,440526	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108434
ZMA. MOLL. 114454	NMNL	06.05.1961	The Netherlands, Zeeland, Aardenburg	51,259988	3,440526	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114454
ZMA. MOLL. 114425	NMNL	02.06.1961	The Netherlands, Zeeland, Grevelingen	51,770309	3,863317	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114425
47061	ZMH	May 1962	Italy, Pineto, Pescara	42,46368972	14,232655	found alive	
RMNH. MOL. 34063	NMNL	30.05.1962	Italy, Friuli–Venezia Giulia, Grado	45,680035	13,372924	unknown	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.34063
ZMA. MOLL. 108522	NMNL	June 1962	The Netherlands, North Holland, Sloterdijk	52,396274	4,861736	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108522
RGM. 812844	NMNL	Oct-Nov 1962	The Netherlands, Zeeland, Ouwerkerk	51,633807	3,939132	unknown	http://data.biodiversitydata.nl/naturalis/specimen/RGM.812844
ZMA. MOLL. 222799	NMNL	23.03.1963	The Netherlands, North Holland, Amsterdam	52,385135	4,844839	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.222799
ZMA. MOLL. 108439	NMNL	Apr 1963	The Netherlands, North Holland, Amsterdam	52,42979	4,737103	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108439

ZMA. MOLL. 108470	NMNL	Apr 1963	The Netherlands, Zeeland, Domburg	51,550543	3,456949	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108470
ZMA. MOLL. 114458	NMNL	Apr 1963	The Netherlands, Noord Holland, Amsterdam	52,376092	4,902026	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114458
ZMA. MOLL. 108460	NMNL	27.04.1963	The Netherlands, Zeeland, De Kauter near Nieuw Namen	51,293493	4,155848	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108460
ZMA. MOLL. 114467	NMNL	Oct 1963	The Netherlands, Zeeland	51,370774	3,366116	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114467
2470646	NHML	28.07.1964	England, Essex, Paglesham, Burnham-on-Crouch, River Roach	51,624777	0,815487	unknown	http://data.nhm.ac.uk/object/c5251449-18cb-49cc-8675-18915b536e69
47062	ZMH	1965	Denmark, Oddesund bay	56,5999175	8,588028889	found dead	
47055	ZMH	Aug 1966	Denmark, Klintebjaerg, Odense Fjord	55,48333333	10,45	found dead	
47068	ZMH	1967	Croatia, Kvarner Bay, Mali Losinj	44,57124639	14,44344472	found alive	
33658	ZMH	23.04.1967	Germany, Schleswig-Holstein, Helgoland, Dune	54,19191833	7,919499444	unknown	
Mo 1356	ZMK	21.09.1967	Italy, Ischia, Porto d'Ischia, zoological Institute, beach with sand, gravel and stones	40,74277833	13,94121028	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 1151	ZMK	22.09.1967	Italy, Campania, Lago d'Averno bear Naples, inside of the Jupiter temple ruins	40,84232056	14,07764889	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 1069	ZMK	25.09.1967	Italy, Sardinia, Cagliari, near the zoological Institute, beach	39,21297028	9,107150833	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 1081	ZMK	25.09.1967	Italy, Sardinia, Cagliari, near the zoological Institute, beach	39,21297028	9,107150833	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 1243	ZMK	27.09.1967	Italy, Sardinia, Cagliari, near the zoological Institute, beach	39,21297028	9,107150833	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 1172	ZMK	02.10.1967	Italy, Sardinia, Nora, sandy beach	38,99064694	9,013971944	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 1376	ZMK	03.10.1967	Italy, Sardinia, Oristano, sandy beach, "Kjökkenmoddinger" near fisher village	39,90130861	8,487539167	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 1055	ZMK	05.10.1967	Italy, Sardinia, Cagliari, near the zoological Institute	39,21297028	9,107150833	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
47057	ZMH	Jul 1968	Croatia, Pula, Fishing Harbor	44,86666667	13,81666667	found dead	
33668	ZMH	Aug 1968	Germany, Schleswig-Holstein, Amrum	54,71213694	8,357631389	found dead	
Mo 4514	ZMK	Sep 1968	France, Gironde, Cap Ferret	44,66276194	−1,259307778	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 4541	ZMK	Sep 1968	France, Gironde, Cap Ferret	44,66276194	−1,259307778	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 4542	ZMK	Sep 1968	France, Gironde, Cap Ferret	44,66276194	−1,259307778	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 4540	ZMK	06.09.1968	France, Gironde, Arcachon, Le Moulleau	44,64722333	−1,199311944	unknown	http://zmk.sesam.senckenberg.de/page/index.htm
ZMA. MOLL. 108562	NMNL	1969	The Netherlands, Zeeland, Yerseke	51,519109	4,017162	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108562
ZMA. MOLL. 2503672	NMNL	01.01.1970	The Netherlands, North Brabant, Bergen-op-Zoom	51,511459	4,220558	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.2503672
ZMA. MOLL. 2503673	NMNL	01.01.1970	The Netherlands, North Holland, Texel	53,185393	4,85608	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.2503673

ZMA. MOLL. 2503676	NMNL	01.01.1970	The Netherlands, North Brabant, Bergen-op-Zoom	51,511459	4,220558	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.2503676
ZMA. MOLL. 114460	NMNL	01.01.1970	The Netherlands, Zeeland, Vlissingen	51,439545	3,601125	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114460
ZMA. MOLL. 2503674	NMNL	01.01.1970	The Netherlands, North Holland, Texel	53,185393	4,85608	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.2503674
ZMA. MOLL. 2503678	NMNL	01.01.1970	The Netherlands, North Holland, Texel	53,185393	4,85608	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.2503678
ZMA. MOLL. 108550	NMNL	01.01.1970	The Netherlands, Zeeland, Vlissingen	51,439545	3,601125	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108550
ZMA. MOLL. 2503701	NMNL	01.01.1970	The Netherlands, North Brabant, Bergen-op-Zoom	51,511459	4,220558	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.2503701
ZMA. MOLL. 114468	NMNL	10.07.1970	The Netherlands, Friesland, Terschelling	53,351995	5,151671	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114468
Mo 6110	ZMK	1971	Sweden, Bohuslän	59,08970694	11,23455028	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 6114	ZMK	1971	Germany, Schleswig-Holstein, Sylt, Hörnum	54,79363194	8,294999722	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
87482	ZMH	1972	Greece, Malacondas, Eubbda	38,4	23,76666667	found alive	
RMNH. MOL. 95432	NMNL	1972	North Sea, Klaverbank	52,512952	5,070051	unknown	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.95432
87130	ZMH	14.04.1973	Italy, Muggia, south of Trieste	45,60416667	13,7675	found alive	
87122	ZMH	15.04.1973	Croatia, Porec	45,23026333	13,59834472	found dead	
Mo 12/11	ZMK	ca. 1974	Germany, Schleswig-Holstein, North Sea, west coast	55,05600889	8,41318	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
ZMA. MOLL. 108489	NMNL	01.03.1975	The Netherlands, North Holland, IJmuiden port	52,463805	4,532315	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108489
Biv 0496	ZSRO	10.07.1975	Bulgaria, Wesseli, stony coast	42,32882167	27,74235444	found alive	http://zsro.sesam.senckenberg.de/page/index.htm
II-E/8238	DMM	01.03.1976	Germany, Mecklenburg-Vorpommern, Greifswald near Loissin	54,09436	13,493969	found dead	
Biv 0497	ZSRO	11.06.1977	Bulgaria, Burgas, Lozenec	42,22250389	27,78704778	found dead	http://zsro.sesam.senckenberg.de/page/index.htm
Biv 0498	ZSRO	14.07.1997 & 20.07.1997	Sweden, Västra Götaland County, Fiskgebäckskil, Gulmarnfjord	58,24758639	11,45367389	unknown	http://zsro.sesam.senckenberg.de/page/index.htm
ZMA. MOLL. 112557	NMNL	18.07.1977	The Netherlands, Friesland, Terschelling	53,351995	5,151671	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.112557
Biv 0515	ZSRO	23.08.1997 – 06.09.1997	Spain, Ibiza, beaches „Cala Lleyna" and "Cala Nova"	39,02087944	1,59387	found dead	http://zsro.sesam.senckenberg.de/page/index.htm
II-E/9107	DMM	17.05.1981	Denmark, Syddanmark, Fanø, Rømø, washed ashore	55,46863	8,355612	found dead	
ZMA. MOLL. 105954	NMNL	01.04.1982	France , Occitania, Languedoc-Roussillon, La Tamarissiere	43,288096	3,437257	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.105954
47063	ZMH	Jul 1982	France, Bretagne	48,64314083	−1,586818056	found dead	

ZMA. MOLL. 112555	NMNL	29.07.1982	The Netherlands, Friesland, Terschelling	53,351995	5,151671	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.112555
ZMA. MOLL. 112671	NMNL	11.08.1982	The Netherlands, Friesland, Terschelling	53,351995	5,151671	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.112671
309308	SMF	07.08.1984	Sweden, in front of Stavanger	58,979354	5,759543	unknown	https://search.senckenberg.de/aquila-public-search/search
309392	SMF	13.08.1984	Germany, Schleswig-Holstein, Helgoland, "Tiefe Rinne"	54,133686	7,885927	unknown	https://search.senckenberg.de/aquila-public-search/search
ZMA. MOLL. 15077	NMNL	18.06.1985	France, Bretagne, Les Rosaires, sta. pel85/18	48,567868	−2, 764934	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.15077
Biv 0507	ZSRO	Apr 1986	Germany, Schleswig-Holstein, Amrum	54,71264444	8,357571667	unknown	http://zsro.sesam.senckenberg.de/page/index.htm
Biv 3166	ZSRO	Apr 1986	Germany, Schleswig-Holstein, Amrum	54,71264444	8,357571667	unknown	http://zsro.sesam.senckenberg.de/page/index.htm
Biv 3167	ZSRO	1987/1988	Bulgaria, Black Sea coast	43,7226275	28,59613694	found dead	http://zsro.sesam.senckenberg.de/page/index.htm
7866	ZMH	1989	France, Le Dramont	43,41640611	6,837100833	found dead	
ZMA. MOLL. 126064	NMNL	Sep 1990	The Netherlands, Zeeland, Cadzand, beach	51,368984	3,366785	found dead	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.126064
Biv 0516	ZSRO	21.04.1992 & 23.04.1992	Turkey, Mugla, beach between Iztuzu and Dalyanagzi	36,80092778	28,60202389	found dead	http://zsro.sesam.senckenberg.de/page/index.htm
II-E/12234	DMM	24.04.1994	Denmark, Syddanmark, Fanø, wadden shore	55,4688	8,359153	found dead	
II-E/12244	DMM	24.04.1994	Denmark, Syddanmark, Rømø	55,21102	8,51355	found dead	
II-E/15037	DMM	May-Sep 1994	Norway, Nordland, Moskenesøy, shore near Fredwang, Lofoten	67,82533	12,808977	found dead	
ZMA. MOLL. 90457	NMNL	11.08.1994	The Netherlands, Zeeland, Bruinisse	52,6167	4,6333	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.90457
II-E/12539	DMM	28.07.1995	Germany, Schleswig-Holstein, Helgoland, "Tiefe Rinne"	54,15641	7,929456	found dead	
47053	ZMH	1997	Germany, Niedersachsen, Cuxhaven, Elbe estuary	53,89184639	8,688039722	found dead	

Table S3: Details of *Ostrea edulis* of the 21st century, chronologically ordered.

catalogue number	facility	sampling date	sampling location	latitude	longitude	status	publication
ZMA. MOLL. 108558	NMNL	27.07.2000	The Netherlands, Zeeland, Yersche Bank	51,518893	4,017248	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.108558
Biv 3169	ZSRO	2002	Germany, Niedersachsen, Juist, North Sea	53,66267333	6,850921111	found dead	http://zsro.sesam.senckenberg.de/page/index.htm
ZMA. MOLL. 13619	NMNL	04.05.2002	Greece, North Aegean, Lesbos, Skala Kalloni	39,197115	26,172113	unknown	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.13619
132136	ZMH	Aug 2003	Italy, Tortoli, beach	39,91666667	9,683333333	found dead	
132134	ZMH	Jul 2007	France, Frontignan, beach	43,43333333	3,766666667	found dead	
132137	ZMH	Jul 2007	France, Port-la-Nouvelle, beach	43	3,05	found dead	
/	ZMH	Feb 2008	Germany, Schleswig-Holstein, Sylt, List, beach	55,01666667	8,433333333	found dead	

Biv 3173	ZSRO	11.02.2008	Germany, Niedersachsen, Wangerooge	53,78712	7,845998333	found dead	http://zsro.sesam.senckenberg.de/page/index.htm
132135	ZMH	Jul 2008	Italy, Lignano, beach	45,66666667	13,11666667	found dead	
IM 2009-7744	MNHN	2009	North-East Atlantic coast	48,253724	−7,971872	unknown	http://coldb.mnhn.fr/catalognumber/mnhn/im/2009-7744
IM 2009-7745	MNHN	2009	North-East Atlantic coast	48,253724	−7,971872	unknown	http://coldb.mnhn.fr/catalognumber/mnhn/im/2009-7745
IM 2009-7746	MNHN	2009	North-East Atlantic coast	48,253724	−7,971872	unknown	http://coldb.mnhn.fr/catalognumber/mnhn/im/2009-7746
Biv 3227	ZSRO	May 2009	Spain, Atlantic coast	37,16203333	−7,38564	found dead	http://zsro.sesam.senckenberg.de/page/index.htm
Biv 4282	ZSRO	04.10.2009	Italy, Friaul, Grado, drift line	45,68194972	13,43858611	found dead	http://zsro.sesam.senckenberg.de/page/index.htm
RMNH. MOL. 128149	NMNL	22.08.2010	Ukraine, Black Sea, Autonomous Republic of Crimea, Evpatoria district, Popivka	45,296323	33,028752	unknown	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.128149
IM 2009-30856	MNHN	2012-2013	Africa, Tunisia, island Djerba, Golf of Gabes	33,699248	10,760795	unknown	http://coldb.mnhn.fr/catalognumber/mnhn/im/2009-30856
IM 2009-30857	MNHN	2012-2013	Africa, Tunisia, island Djerba, Golf of Gabes	33,699248	10,760795	unknown	http://coldb.mnhn.fr/catalognumber/mnhn/im/2009-30857
Mo 8801	ZMK	08.10.2017	Germany, Schleswig-Holstein, Baltic Sea, short beach between Bülk and Strande, beneath sand on the shore	54,44451667	10,18145083	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8802/5	ZMK	13.-18.08.2017	France, Hérault, beach between Sète and Marseillan, beach wall separating the lake from the Sea, infront of camping site	43,33401222	3,575663333	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8803/2	ZMK	13.-18.08.2017	France, Hérault, beach between Sète and Marseillan, beach wall separating the lake from the Sea, infront of camping site	43,33401222	3,575663333	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8900/19	ZMK	16.-19.10.2017	Germany, Schleswig-Holstein, Sylt, Rantum, beach infront of the Café “Strandmuschel”, drift line at low tide	54,84715194	8,285665278	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8901/1	ZMK	16.-19.10.2017	Germany, Schleswig-Holstein, Sylt, Rantum, beach infront of the Café “Strandmuschel”, drift line at low tide	54,84715194	8,285665278	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8934/1	ZMK	18.09.2017	Ireland, Connacht, Galway, bought from Kelly Oysters	53,19125611	−8,954923611	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8935/1	ZMK	18.09.2017	Ireland, Connacht, Galway, bought from Kelly Oysters	53,19125611	−8,954923611	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8936/1	ZMK	18.09.2017	Ireland, Connacht, Galway, bought from Kelly Oysters	53,19125611	−8,954923611	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8937/1	ZMK	18.09.2017	Ireland, Connacht, Galway, bought from Kelly Oysters	53,19125611	−8,954923611	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8938/1	ZMK	18.09.2017	Ireland, Connacht, Galway, bought from Kelly Oysters	53,19125611	−8,954923611	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8939/1	ZMK	18.09.2017	Ireland, Connacht, Galway, bought from Kelly Oysters	53,19125611	−8,954923611	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8940/1	ZMK	18.09.2017	Ireland, Connacht, Galway, bought from Kelly Oysters	53,19125611	−8,954923611	found alive	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8941/1	ZMK	18.09.2017	Ireland, Connacht, Galway, bought from Kelly Oysters	53,19125611	−8,954923611	found alive	http://zmk.sesam.senckenberg.de/page/index.htm

Mo 9075/20	ZMK	16.04.2018	Germany, Schleswig-Holstein, „Hoyerstief“ oyster bed	55,01123333	8,503533333	found dead	http://zmk.sesam.senckenberg.de/page/index.htm
Biv 4507	ZSRO	01.10.2018	Denmark, Jutland, island Fur, Limfjord	56,84072	8,985952	unknown	http://zsro.sesam.senckenberg.de/page/index.htm

Table S4: Details of *Crepidula fornicata* of the 20th century, chronologically ordered.

catalogue number	facility	sampling date	sampling location	latitude	longitude	publication
ZMA. MOLL. 337308	NMNL	1920	Wales, Anglesey, Irish Sea	53,430015	−4, 425759	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.337308
ZMA. MOLL. 114306	NMNL	05.09.1926	The Netherlands, North Holland, Camperduin-Petten	52,770647	4,662477	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114306
ZMA. MOLL. 114313	NMNL	Jun 1927	The Netherlands, South Holland, Den Haag, south Gravenhage	52,060823	4,282565	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114313
3500869	NHML	1928 - 1873	Africa	34,71638781	−6, 451831	https://doi.org/10.5519/0002965
RMNH. MOL. 180644	NMNL	14.09.1929 - 15.09.1929	England, Essex, Davercourt	51,923302	1,269632	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.180644
ZMA. MOLL. 2503658	NMNL	Dec 1929	The Netherlands, Zeeland, Yerseke	51,518893	4,017248	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.2503658
RMNH. MOL. 180636	NMNL	1930	England, Island Wight, Ryde	50,746737	−1, 242675	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.180636
2680033	NHML	Aug 1930	Europe	52,258353	4,374702	https://doi.org/10.5519/0002965
RMNH. MOL. 180660	NMNL	15.08.1930	England, Essex, Davercourt Bay	51,923302	1,269632	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.180660
ZMA. MOLL. 114274	NMNL	Nov 1930	The Netherlands, Zeeland, Oosterschelde	51,566338	3,948963	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114274
ZMA. MOLL. 337317	NMNL	05.11.1930	England, Eling near Southampton	50,909967	−1, 482299	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.337317
RMNH. MOL. 180672	NMNL	27.07.1931 - 01.08.1931	England, Hampshire, the Solent, Warren beach	50,767171	−1, 415843	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.180672
ZMA. MOLL. 114278	NMNL	Feb 1933	The Netherlands, Zeeland, Yerseke	51,518893	4,017248	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114278
ZMA. MOLL. 114279	NMNL	Feb 1933	The Netherlands, Zeeland, Zierikzee	51,667267	3,876784	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114279
ZMA. MOLL. 114260	NMNL	22.06.1933	The Netherlands, Zeeland, Yerseke	51,518893	4,017248	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114260
3521197	NHML	Jul 1934	Europe	52,258353	4,374702	https://doi.org/10.5519/0002965
RMNH. MOL. 180632	NMNL	22.04.1935 - 26.04.1935	England, Dorset, Bridport beach	50,715236	−2, 782939	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.180632
ZMA. MOLL. 114287	NMNL	May 1935	The Netherlands, Zeeland, Westenschouwen	51,674538	3,695683	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114287
ZMA. MOLL. 114270	NMNL	Jul 1935	The Netherlands, Zeeland, Bruinisse	51,667427	4,095682	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114270
ZMA. MOLL. 114261	NMNL	Oct 1935	The Netherlands, Zeeland, Yerseke	51,518893	4,017248	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114261

RMNH. MOL. 180630	NMNL	Jan 1936	England, Eddystone near Plymouth	50,18	−4.265	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.180630
ZMA. MOLL. 114283	NMNL	Feb 1936	The Netherlands, Zeeland, Bruinisse	51,667427	4,095682	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114283
ZMA. MOLL. 114262	NMNL	17.08.1936	The Netherlands, Zeeland, Yerseke	51,518893	4,017248	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114262
ZMA. MOLL. 114296	NMNL	Sep 1936	The Netherlands, Zeeland, Yerseke	51,519109	4,017162	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114296
ZMA. MOLL. 159393	NMNL	28.08.1937	The Netherlands, Zeeland, Yerseke	51,518893	4,017248	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.159393
ZMA. MOLL. 337339	NMNL	1938	Germany, Niedersachsen, Juist	53,661046	6,852295	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.337339
ZMA. MOLL. 114284	NMNL	Feb 1938	The Netherlands, Zeeland, Vlissingen beach	51,439545	3,601125	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114284
ZMA. MOLL. 114259	NMNL	May 1938	The Netherlands, Zeeland, Veere	51,518893	4,017248	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114259
ZMA. MOLL. 114276	NMNL	04.05.1938	The Netherlands, Zeeland, Veere	51,564077	3,639758	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114276
ZMA. MOLL. 114326	NMNL	26.09.1938	The Netherlands, North Holland, Den Oever	52,934895	4,999602	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114326
ZMA. MOLL. 114323	NMNL	1939	The Netherlands, North Holland, Den Oever	52,934895	4,999602	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114323
ZMA. MOLL. 114330	NMNL	1939	The Netherlands, North Holland, Den Oever	52,934895	4,999602	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114330
ZMA. MOLL. 114290	NMNL	1939	The Netherlands, Zeeland, Terneuzen	51,342849	3,81423	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114290
ZMA. MOLL. 114320	NMNL	1939	The Netherlands, North Holland, Den Oever	52,934895	4,999602	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114320
RMNH. MOL. 180637	NMNL	14.04.1939	England, Dorset, Studland beach	50,679655	−1, 949412	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.180637
RMNH. MOL. 180631	NMNL	16.04.1939	England, Dorset, Swanage beach	50,644649	−1, 946178	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.180631
RMNH. MOL. 180653	NMNL	20.04.1939	England, Dorset, Studland beach	50,679655	−1, 949412	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.180653
ZMA. MOLL. 114263	NMNL	Nov 1940	The Netherlands, Zeeland, Yerseke, Oosterschelde	51,519109	4,017162	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114263
ZMA. MOLL. 114317	NMNL	25.01.1941	The Netherlands, North Holland, Zandvoort	52,244756	4,426475	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114317
Mo 8887/11	ZMK	Apr 1943	France, Bretagne, Penmarch	47,84173583	-4,3512725	http://zmk.sesam.senckenberg.de/page/index.htm
ZMA. MOLL. 114286	NMNL	Jul 1943	The Netherlands, Zeeland, Ouwerkerk	51.633807	3,939132	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114286
ZMA. MOLL. 114285	NMNL	08.10.1946	The Netherlands, Zeeland, Schakerloo	51,890688	4,324347	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114285

RMNH. MOL. 71442	NMNL	1948	The Netherlands, South Holland, between Zandvoort and Noordwijk	52,244756	4,426475	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.71442
Mo 8897/1	ZMK	Jul 1948	Germany, Schleswig-Holstein, Sylt, Königshafen	55,05824306	8,416306944	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8886/6	ZMK	Aug 1948	Germany, Schleswig-Holstein, Sylt, beach on northern tip	55,05846278	8,416814722	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8893/6	ZMK	Aug 1948	Germany, Schleswig-Holstein, Sylt, List	55,05824306	8,416306944	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 807/13	ZMK	Aug/Sep 1948	Germany, Schleswig-Holstein, Sylt, Königshafen	55,05824306	8,416306944	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8883/11	ZMK	Aug/Sep 1948	Germany, Schleswig-Holstein, Sylt, Königshafen	55,05824306	8,416306944	http://zmk.sesam.senckenberg.de/page/index.htm
Mo 8888/29	ZMK	Aug/Sep 1948	Germany, Schleswig-Holstein, Sylt, southern beach on northern tip	55,04592556	8,395786111	http://zmk.sesam.senckenberg.de/page/index.htm
RMNH. MOL. 5009348	NMNL	Sep 1948	The Netherlands, Friesland, Schiermonnikoog	53,461176	6,113495	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.5009348
Mo 8898/1	ZMK	Sep 1948	Germany, Schleswig-Holstein, Sylt, Königshafen/List	55,05824306	8,416306944	http://zmk.sesam.senckenberg.de/page/index.htm
2448552	NHML	25.09.1948	Europe	52,258353	4,374702	https://doi.org/10.5519/0002965
RMNH. MOL. 5005343	NMNL	1949	The Netherlands, South Holland, Scheweningen	52,08238	4,233919	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.5005343
ZMA. MOLL. 114294	NMNL	Jul 1949	The Netherlands, Zeeland, Rammekens	51,458446	3,662481	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114294
ZMA. MOLL. 114291	NMNL	18.08.1949	The Netherlands, Zeeland, Terneuzen	51,342849	3,81423	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114291
ZMA. MOLL. 114282	NMNL	20.08.1949	The Netherlands, Zeeland, coast near Othene	51,334876	3,857412	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114282
ZMA. MOLL. 114273	NMNL	26.08.1949	The Netherlands, Zeeland, Domburg	51,550543	3,456949	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114273
ZMA. MOLL. 114268	NMNL	31.08.1949	The Netherlands, Zeeland, Terneuzen	51,342849	3,81423	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114268
ZMA. MOLL. 114312	NMNL	18.07.1950	The Netherlands, Friesland, Terschelling	53,351995	5,151671	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114312
ZMA. MOLL. 114300	NMNL	19.07.1950	The Netherlands, North Holland, Texel	53,076115	4,894017	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114300
RMNH. MOL. 180650	NMNL	03.08.1950 - 16.08.1950	Spain, Girona, Cadaques Bay	42,27443	3,280734	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.180650
RMNH. MOL. 180647	NMNL	07.08.1950	Spain, Girona, Playa d'en Pere Fet near Cadaques	42,285458	3,285006	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.180647
ZMA. MOLL. 114265	NMNL	26.08.1950	The Netherlands, Zeeland, Kistersinlaag, south of Schouwen	51,68055556	3,835833333	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114265
ZMA. MOLL. 114295	NMNL	28.08.1950	The Netherlands, Zeeland, Zierikzee, Kistersinlaag	51,68055556	3,835833333	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114295
ZMA. MOLL. 114292	NMNL	01.01.1951	The Netherlands, Zeeland, Wemeldinge	51,527488	3,972102	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114292
2448553	NHML	24.03.1951	Europe	52,258353	4,374702	https://doi.org/10.5519/0002965
Mo 8858/3	ZMK	Mar/Apr 1951	Germany, Schleswig-Holstein, Föhr, beach	54,75553861	8,533458611	http://zmk.sesam.senckenberg.de/page/index.htm

ZMA. MOLL. 114277	NMNL	01.08.1953	The Netherlands, Zeeland, Schelphoek	51,701037	3,793613	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114277
ZMA. MOLL. 114289	NMNL	01.11.1953	The Netherlands, Zeeland, Zierikzee, on mussel beds at Boerenweg	51,667267	3,876784	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114289
RMNH. MOL. 71443	NMNL	16.05.1954	The Netherlands, Friesland, north west of Nieuwe Bildtzijl near Ferwerderadiel, Noorderleeg	53,319647	5,726609	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.71443
Mo 8895/5	ZMK	Jun 1954	Germany, Schleswig-Holstein, Sylt, northern tip	55,05846278	8,416814722	http://zmk.sesam.senckenberg.de/page/index.htm
RMNH. MOL. 71441	NMNL	19.07.1954	The Netherlands, Friesland, Terschelling, near Hoorn	53,351995	5,151671	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.71441
ZMA. MOLL. 114318	NMNL	12.08.1954	The Netherlands, Groningen, Rottumeroog	53,539153	6,56481	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114318
ZMA. MOLL. 127413	NMNL	1955	The Netherlands, North Holland, Texel	53,076115	4,894017	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.127413
ZMA. MOLL. 125418	NMNL	22.09.1955	The Netherlands, South Holland, Oostvoorne, beach	51,922791	4,038344	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.125418
2754545	NHML	02.04.1956	Europe	52,258353	4,374702	https://doi.org/10.5519/0002965
ZMA. MOLL. 125495	NMNL	25.07.1957	The Netherlands, South Holland, Oostvoorne	51,922791	4,038344	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.125495
31468	SNSD	04.12.1957	Germany, Schleswig-Holstein, Föhr, Wyk	54,680423	8,536406	
ZMA. MOLL. 114281	NMNL	Jun 1959	The Netherlands, Zeeland, Wemeldinge	51,527488	3,972102	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114281
ZMA. MOLL. 114301	NMNL	Aug 1960	The Netherlands, Friesland, Terschelling	53,351995	5,151671	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114301
RMNH. MOL. 5009347	NMNL	Jul 1961	The Netherlands, Groningen, Eems near Tolen	53,40783	6,874424	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.5009347
ZMA. MOLL. 114303	NMNL	Jul 1961	The Netherlands, Friesland, Terschelling, Oosterend	53,351995	5,151671	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114303
2464165	NHML	24.08.1961	Europe	52,258353	4,374702	https://doi.org/10.5519/0002965
ZMA. MOLL. 126913	NMNL	08.06.1962	The Netherlands, North Holland, IJmuiden	52,440319	4,556404	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.126913
ZMA. MOLL. 114324	NMNL	Jul 1964	The Netherlands, North Holland, Zandvoort	52,40027	4,53871	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114324
2464166	NHML	28.07.1964	Europe	52,258353	4,374702	https://doi.org/10.5519/0002965
2464167	NHML	30.07.1964	Europe	52,258353	4,374702	https://doi.org/10.5519/0002965
5772	DMM	11.10.1968 - 16.10.1968	Sweden, Bohuslän, Kristineberg, zoolog. station, Gullmarnfjord	58,249749	11,444378	
ZMA. MOLL. 125555	NMNL	28.05.1969	The Netherlands, Friesland, Schiermonnikoog	53,461176	6,113495	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.125555
ZMA. MOLL. 114272	NMNL	1970	The Netherlands, Zeeland, Domburg	51,550543	3,456949	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114272
ZMA. MOLL. 114307	NMNL	May 1970	The Netherlands, Zeeland	51,370774	3,366116	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114307

ZMA. MOLL. 114319	NMNL	29.08.1970	The Netherlands, North Holland, Ijmuiden, south pier	52,440319	4,556404	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114319
ZMA. MOLL. 114280	NMNL	Jul 1973	The Netherlands, North Holland, Texel	53,076115	4,894017	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114280
ZMA. MOLL. 114304	NMNL	28.02.1974	The Netherlands, North Holland, Texel	53,076115	4,894017	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114304
ZMA. MOLL. 114322	NMNL	29.09.1974	The Netherlands, North Holland, Texel	53,076115	4,894017	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114322
ZMA. MOLL. 114298	NMNL	Jul 1975	The Netherlands, Friesland, Terschelling, Lies, shore	53,377399	5,323762	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114298
ZMA. MOLL. 2286479	NMNL	08.07.1975	France, Normandie, Cotentin, La Madeleine	49,408625	−1,166815	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.2286479
ZMA. MOLL. 114258	NMNL	Nov 1975	The Netherlands, Zeeland, Yerseke, between oysters	51,518893	4,017248	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114258
ZMA. MOLL. 35299	NMNL	01.07.1976	England, Island Herm	49,483628	−2,448154	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.35299
ZMA. MOLL. 114271	NMNL	14.12.1976	The Netherlands, Zeeland, Wemelding lock, Oosterschelde	51,52159	4,003342	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114271
RMNH. MOL. 110068	NMNL	05.02.1977	The Netherlands, Zeeland, Schouwen-Duivenland, 3.5km west of Kerkwerve Flauwers	51,677802	3,857428	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.110068
308711	SMF	22.06.1977	Germany, Schleswig-Holstein, Helgoland, „Tiefe Rinne“, FS-30 Ku	54,15640983	7,929455885	https://search.senckenberg.de/aquila-public-search/search
ZMA. MOLL. 114329	NMNL	18.07.1977	The Netherlands, Friesland, Terschelling	53,351995	5,151671	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114329
15044	DMM	01.05.1978	Belgium, Westfladern, Oostduinkerke	51,128162	2,64826	
9118	DMM	17.05.1981	Denmark, Syddanmark, Rømø	55,210823	8,515829	
ZMA. MOLL. 114311	NMNL	23.07.1982	The Netherlands, Friesland, Terschelling	53,351995	5,151671	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114311
ZMA. MOLL. 114328	NMNL	14.04.1984	The Netherlands, Friesland, Terschelling	53,351995	5,151671	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114328
ZMA. MOLL. 114314	NMNL	21.04.1984	The Netherlands, Friesland, Terschelling	53,351995	5,151671	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114314
ZMA. MOLL. 114327	NMNL	24.07.1985	The Netherlands, Friesland, Terschelling	53,351995	5,151671	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114327
ZMA. MOLL. 114316	NMNL	13.08.1986	The Netherlands, Friesland, Terschelling	53,351995	5,151671	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114316
3522627	NHML	14.10.1987	Europe	52,258353	4,374702	https://doi.org/10.5519/0002965
310196	SMF	11.07.1988	England, estuary of the Thames	51,616628	0,958646	https://search.senckenberg.de/aquila-public-search/search
310204	SMF	11.07.1988	England, northern estuary of the Thames	51,509915	0,545285	https://search.senckenberg.de/aquila-public-search/search
310211	SMF	11.07.1988	England, Norfolk, in front of Great-Yarmouth	52,66408	1,73045	https://search.senckenberg.de/aquila-public-search/search
310217	SMF	11.07.1988	England, Norfolk, in front of Cromer	52,943656	1,233888	https://search.senckenberg.de/aquila-public-search/search
ZMA. MOLL. 110	NMNL	10.09.1990	Denmark, Nordjylland, Lyngsa, Kattegat	57,248099	10,550256	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.110

12634	DMM	Oct 1990	Germany, Schleswig-Holstein, Helgoland	54,190766	7,864945	
11986	DMM	06.11.1990 - 08.11.1990	Germany, Schleswig-Holstein, Sylt, List, Königshafen	55,027979	8,428702	
11987	DMM	06.11.1990 - 08.11.1990	Germany, Schleswig-Holstein, Sylt, List, Königshafen	55,027979	8,428702	
12648	DMM	Jul 1991	England, Brighton beach, English channel	50,821446	−0,155874	
13854	DMM	25.08.1991	France, Bretagne, Carnac, Bay of Biscay	47,583477	−3,103621	
ZMA. MOLL. 114275	NMNL	1993	The Netherlands, Zeeland, Grevelingenmeer	51,770309	3,863317	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114275
ZMA. MOLL. 114310	NMNL	Aug 1993	The Netherlands, North Holland, Egmond-aan-Zee	52,62007	4,617197	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114310
ZMA. MOLL. 90454	NMNL	11.08.1994	The Netherlands, Zeeland, Bruinisse	51,667427	4,095682	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.90454
36622	SNSD	Oct 1994	Belgium, Kooksijde, on beach after storm	51,110258	2,603278	
ZMA. MOLL. 114302	NMNL	Jun 1996	The Netherlands, Zeeland, Brouwershaven, Grevelingenmeer	51,742035	3,87452	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114302
12708	DMM	01.06.1996 - 07.06.1996	Sweden, Bohuslän, Kristineberg, zoolog. station, Gullmarnfjord	58,249791	11,444581	
40488	SNSD	1998	Germany, Schleswig-Holstein, Sylt, List	55,034395	8,406005	
RMNH. MOL. 32695	NMNL	24.08.1998	The Netherlands, South Holland, Katwijk	52,185098	4,372017	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.32695
40487	SNSD	16.10.1998	Germany, Schleswig-Holstein, Sylt, List	55,034395	8,406005	
13853	DMM	14.09.1999	France, Bretagne, Regnéville-sur-Mer, English channel	48,99766	−1,558008	

Table S5: Details of *Crepidula fornicata* of the 21st century, chronologically ordered.

44739	SNSD	18.06.2002	Germany, Schleswig-Holstein, Sylt, northern tip	55,058391	8,416378	
44740	SNSD	18.06.2002	Germany, Schleswig-Holstein, Sylt, Rantum, West beach	54,795743	8,281785	
13855	DMM	29.04.2003	France, Bretagne, Locmariaquer, Pierre Plates beach, Bay of Biscay	47,583729	−2,989559	
46038	SNSD	24.07.2003	Germany, Schleswig-Holstein, Helgoland, marine station	54,183711	7,888358	
46013	SNSD	24.07.2003	Germany, Niedersachsen, Borkum	53,591959	6,638298	
13856	DMM	20.04.2004	France, Languedoc, Fleury d'Aude	42,435616	3,174705	
ZMA. MOLL. 114293	NMNL	03.01.2005	The Netherlands, North Holland, Hondsbossche Zeewering	52,739182	4,641283	http://data.biodiversitydata.nl/naturalis/specimen/ZMA.MOLL.114293
13857	DMM	20.05.2005	France, Nouvelle-Aquitaine, Ile d'Oleron, La Cotiniere, Bay of Biscay	46,048033	−1,410694	
RMNH. MOL. 102104	NMNL	11.02.2006	The Netherlands, South Holland, Katwijk, north of river Rhine, after a few cold days	52,211486	4,398314	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.102104
RMNH. MOL. 111802	NMNL	06.09.2008	The Netherlands, Zeeland, coast between de Roggenplaat and Mariaanshoofd, Oosterschelde	51,566338	3,948963	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.111802
RMNH. MOL. 112089	NMNL	17.10.2008	The Netherlands, Zeeland, Yerseke, Oosterschelde, market oysters from the Oost company	51.495	4,053444444	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.112089

RMNH. MOL. 126055	NMNL	03.07.2010	The Netherlands, Zeelands, near Terneuzen, Westerschelde	51,454344	3,430329	http://data.biodiversitydata.nl/naturalis/specimen/RMNH.MOL.126055
Mo 8902/21	ZMK	16.10.2017-19.10.2017	Germany, Schleswig-Holstein, Sylt, Rantum, in front of Café „Strandmuschel“, beach on low tide	54,84715194	8,285665278	http://zmk.sesam.senckenberg.de/page/index.htm

Table S6: Detailed results of the linear regression tests from the data set of *Ostrea edulis*.

	Estimated coefficient	**Standard error**	**t-value**	**p-value**
complete data base				
intercept	−32.21323	34.14796	−0.943	0.347
year	0.01958	0.01766	1.109	0.269
Without Möbius oysters (1868 - 1885)				
intercept	−45.13829	52.59289	−0.858	0.392
year	0.02620	0.02704	0.969	0.334

The test was calculated with the complete data set and with the oysters collected by Möbius removed. Provided are estimated coefficients, standard errors, t-values and p-values for collection years as a function of the number of shells collected. Note: high significance = ***; low significance = *; no significance = no asterisks

Table S7: Details of the museum collection material of *O. edulis* we used to generate the historical DNA sequences.

ID	**Museum collection ID**	**Collection date**	**organism**	**sample type**	**sampling site**	**Genbank ID**
KAu180235	Mo 25/1	24.03.1871	*Ostrea edulis*	shell	Germany, west coast of Schleswig-Holstein	following
KAu180257	Mo 58/2	23.05.1877	*Ostrea edulis*	shell	Germany, Sylt, oyster bed ‘Huntje’	[following]
KAu180259	Mo 58/4	23.05.1877	*Ostrea edulis*	shell	Germany, Sylt, oyster bed ‘Huntje’	[following]
KAu180260	Mo 58/5	23.05.1877	*Ostrea edulis*	shell	Germany, Sylt, oyster bed ‘Huntje’	[following]
KAu180264	Mo 56/2	24.05.1877	*Ostrea edulis*	shell	Denmark, West coast (east coast of Rømø Island), oyster bed ‘Tagholm’	[following]
KAu180271	Mo 73/5	22.05.1877	*Ostrea edulis*	shell	Germany, west coast of Schleswig-Holstein, oyster bed ‘Westen Amrum’	[following]
KAu180273	Mo 73/7	22.05.1877	*Ostrea edulis*	shell	Germany, west coast of Schleswig-Holstein, oyster bed ‘Westen Amrum’	[following]
KAu180345	Mo 47/1 right	25.05.1877	*Ostrea edulis*	shell	Denmark, west coast, Reisby-Steert	[following]
KAu180348	Mo 47/4 left	25.05.1877	*Ostrea edulis*	shell	Denmark, west coast, Reisby-Steert	[following]
KAu181756	Mo 106/7	1869	*Ostrea edulis*	shell	France, Mediterranean coast, Toulon, oyster bed ‘la Seyne’	[following]
KAu181758	Mo 106/8	1869	*Ostrea edulis*	shell	France, Mediterranean coast, Toulon, oyster bed ‘la Seyne’	[following]
KAu181759	Mo 106/6	1869	*Ostrea edulis*	shell	France, Mediterranean coast, Toulon, oyster bed ‘la Seyne’	[following]
KAu181760	Mo 106/4	1869	*Ostrea edulis*	shell	France, Mediterranean coast, Toulon, oyster bed ‘la Seyne’	[following]
KAu181764	Mo 99/3 right	1869	*Ostrea edulis*	shell	France, Atlantic coast, la Trinité	[following]
KAu181766	Mo 72/1 right	1870	*Ostrea edulis*	shell	Germany, west coast of Schleswig-Holstein, Amrum, 6 years old	[following]

KAu181768	Mo 75/1 right	1878	*Ostrea edulis*	shell	Germany, west coast of Schleswig-Holstein, oyster bed 'Süden Gröde'	[following]
KAu181769	Mo 39/1 right	1870	*Ostrea edulis*	shell	Germany, west coast of Schleswig-Holstein	[following]
KAu181771	Mo 7/2 left	1879	*Ostrea edulis*	shell	Germany, oyster beds of Schleswig-Holstein, infested by polychaetes	[following]
KAu181772	Mo 7/3 right	1879	*Ostrea edulis*	shell	Germany, oyster beds of Schleswig-Holstein, infested by polychaetes	[following]
KAu181773	Mo 7/4 right	1879	*Ostrea edulis*	shell	Germany, oyster beds of Schleswig-Holstein, infested by polychaetes	[following]
KAu181776	Mo 90/1 right	1869	*Ostrea edulis*	shell	England, English Channel, Hayling Island, near Portsmouth, Hampshire	[following]
KAu181778	Mo 80/2 right	1878	*Ostrea edulis*	shell	the Netherlands, Oosterschelde, market oysters	[following]
KAu181779	Mo 80/3 left	1878	*Ostrea edulis*	shell	the Netherlands, Oosterschelde, market oysters	[following]
KAu181780	Mo 92/1 left	1869	*Ostrea edulis*	shell	England, English Channel, Hayling Island (near Portsmouth, Hampshire), 2 years old	[following]
KAu181784	Mo 98/1 right	1869	*Ostrea edulis*	shell	France, Atlantic coast, la Tremblade (Seudre estuary)	[following]
KAu181825	Mo 88/1 right	1869	*Ostrea edulis*	shell	England, North Sea coast, Thames estuary, Herne Bay	[following]
KAu181828	Mo 91/3 right	1869	*Ostrea edulis*	shell	England, English Channel, Hayling Island (near Portsmouth, Hampshire)	[following]
KAu181829	Mo 91/5 left	1869	*Ostrea edulis*	shell	England, English Channel, Hayling Island (near Portsmouth, Hampshire)	[following]
KAu181830	Mo 91/6 left	1869	*Ostrea edulis*	shell	England, English Channel, Hayling Island (near Portsmouth, Hampshire)	[following]
KAu181832	Mo 91/8 right	1869	*Ostrea edulis*	shell	England, English Channel, Hayling Island (near Portsmouth, Hampshire)	[following]
KAu181833	Mo 51/2 right	1876	*Ostrea edulis*	shell	Germany, west coast of Schleswig-Holstein, oyster beds of Sylt, 'with parasites'	[following]
KAu181842	Mo 97/2 left	1869	*Ostrea edulis*	shell	France, Atlantic coast, Île de Ré	[following]
KAu181844	Mo 97/4 right	1869	*Ostrea edulis*	shell	France, Atlantic coast, Île de Ré	[following]
KAu181845	Mo 97/5 right	1869	*Ostrea edulis*	shell	France, Atlantic coast, Île de Ré	[following]
KAu181846	Mo 97/6 right	1869	*Ostrea edulis*	shell	France, Atlantic coast, Île de Ré	[following]
KAu181848	Mo 97/8 right	1869	*Ostrea edulis*	shell	France, Atlantic coast, Île de Ré	[following]
KAu181849	Mo 102/1 right	1869	*Ostrea edulis*	shell	France, Atlantic coast, Bassin d'Arcachon (Dép. Gironde)	[following]

Table S8: Results of Jost's D pairwise analyses of *O. edulis* populations from sampling sites with more than four sampled individuals. Pairwise p-values are shown in the top triangle (blue font). Values with significance are bolded (p-value < 0.05).

	Wadden Sea	**England**	**Atlantic**	**Mediterranean**
Sample size	20	7	8	4
Haplogroups	NS, WS	NS, NEA, KAu181829, SEA	NEA, SEA	KAu181758, SEA
Jost's D				
Wadden Sea	-	**0.047**	**0.019**	**0.030**
England	**0.166158873**	-	0.316	0.548
Atlantic	**0.296833074**	0.028293051	-	0.613
Mediterranean	**0.283629472**	0.023071138	0.003467137	-

Table S9: Diagnostic SNP's of each haplogroup of *O. edulis*

Position	Reference	haplogroup NS	haplogroup NEA	haplogroup WS	haplogroup SEA
24	G	.	A	A	A
77	A	.	G	G	G
98	T	.	C	C	C
197	C	.	T	T	T
204	A	.	G	G	G
213	G	.	A	A	A
219	C	.	T	T	T
253	G	.	.	.	A
258	G	.	A	A	A
339	C	.	T	T	T
361	T	.	C	C	C
480	C	.	T	T	T
544	G	.	A	A	A
665	G	.	.	.	A
667	A	.	G	G	G
791	T	.	C	C	C
797	C	.	T	T	T
806	G	.	A	A	A
809	A	.	G	G	G
815	A	.	G	G	G
872	A	.	C	C	C
914	A	.	.	.	G
987	A	.	G	G	G
1017	A	.	G	G	G
1096	T	.	C	C	C
1100	A	.	.	G	G
1129	A	.	G	G	G
1135	G	.	A	A	A
1156	T	.	.	C	.
1183	T	.	C	C	C
1213	C	.	.	T	.
1259	G	.	A	A	A
1267	A	.	G	G	G
1288	C	.	T	T	T
1446	A	.	G	G	G
1492	G	.	A	A	A
1508	G	.	.	A	.

1543	A	.	G	G	G
1555	A	.	G	G	G
1637	A	.	G	G	G
1700	A	.	G	G	G
1751	C	.	T	T	T
1756	A	.	G	.	.
1805	A	.	G	G	G
1814	T	.	C	C	C
1862	A	.	G	G	G
1865	A	.	G	G	G
1888	A	.	G	G	G
1910	A	.	G	G	G
1988	T	.	C	C	C
2017	T	.	C	C	C
2024	G	.	A	A	A
2027	A	.	G	G	G
2033	T	.	C	C	C
2036	A	.	G	G	G
2039	A	.	G	G	G
2093	C	.	T	T	T
2096	C	.	.	.	T
2210	A	.	G	G	G
2261	T	.	C	C	C
2312	G	.	.	A	A
2459	C	.	T	T	T
2473	A	.	G	G	G
2557	G	.	A	A	A
2564	G	.	.	A	.
2642	C	.	.	.	T
2657	G	.	.	T	.
2750	A	.	.	.	G
2819	C	.	T	T	T
2879	C	.	T	T	T
2900	A	.	G	G	G
2927	C	.	T	T	T
2987	T	.	.	.	C
2993	T	.	C	C	C
3035	G	.	.	A	.
3074	G	.	A	A	A

3152	G	.	.	A	.
3167	T	.	C	C	C
3182	C	.	.	T	.
3259	T	.	.	.	C
3264	C	.	T	T	T
3342	G	.	C	C	C
3363	C	.	T	T	T
3927	A	.	G	G	G
4026	T	.	.	C	C
4027	A	.	G	G	G
4031	T	.	.	C	C
4058	C	.	T	T	T
4111	G	.	.	.	A
4217	C	.	T	T	T
4243	G	.	A	A	A
4345	G	.	T	T	T
4352	G	.	A	A	A
4366	G	.	A	A	A
4384	T	.	C	C	C
4429	T	.	C	.	.
4557	G	.	.	.	A
4785	A	.	G	G	G
4892	C	.	T	T	.
4902	C	.	T	T	T
4906	A	.	G	G	G
4960	T	.	C	C	C
5090	G	.	A	A	A
5098	A	.	A	G	G
5108	G	.	A	A	A
5165	G	.	A	A	A
5174	A	.	.	G	.
5177	A	.	G	G	G
5219	A	.	G	G	G
5246	T	.	C	C	C
5375	T	.	.	.	C
5396	A	.	G	G	G
5419	C	.	.	.	T
5420	A	.	G	G	G
5423	T	.	C	C	C

5425	G	.	A	A	A
5489	T	.	C	C	C
5495	C	.	T	T	T
5615	A	.	.	.	G
5633	C	.	T	T	T
5650	A	.	G	G	G
5681	G	.	A	A	A
5729	A	.	G	G	G
5783	A	.	.	.	G
5816	T	.	C	C	C
5858	G	.	A	A	A
5885	G	.	.	A	.
5957	A	.	G	G	G
5981	C	.	T	T	T
5990	A	.	G	G	G
6044	A	.	G	G	G
6083	G	.	.	A	.
6152	C	.	T	T	T
6167	G	.	A	A	A
6173	C	.	T	T	T
6182	C	.	.	T	T
6185	G	.	.	.	A
6188	G	.	A	A	A
6379	T	.	C	C	C
6442	G	.	A	A	A
6458	C	.	T	T	T
6461	G	.	A	A	A
6464	T	.	C	C	C
6524	G	.	A	A	A
6593	A	.	T	T	T
6614	T	.	C	C	C
6634	T	.	C	C	C
6649	A	.	G	.	G
6758	A	.	G	G	G
6804	T	.	C	C	C
6879	G	.	A	A	A
6897	A	.	G	G	G
6996	T	.	C	C	.
7005	T	.	C	C	C

7032	A	.	G	G	G
7083	A	.	G	G	G
7130	G	.	A	A	A
7197	A	.	.	.	G
7200	C	.	.	T	.
7242	A	.	G	G	G
7449	A	.	G	G	G
7518	G	.	A	A	A
7528	G	.	A	A	A
7531	G	.	.	A	.
7545	G	.	A	A	A
7569	G	.	A	A	A
7597	C	.	T	T	T
7599	A	.	G	G	G
7603	C	.	T	T	T
7624	C	.	T	T	T
7654	A	.	G	.	.
7778	T	.	C	C	C
7840	C	.	T	T	T
7867	G	.	A	A	A
7954	C	.	T	T	T
8077	A	.	G	G	G
8094	C	.	.	.	T
8148	G	.	A	A	A
8158	G	.	A	A	A
8176	A	.	G	G	G
8293	C	.	T	T	T
8323	C	.	T	T	T
8377	T	.	C	C	C
8383	C	.	T	T	T
8395	G	.	.	A	.
8448	G	.	A	A	A
8449	A	.	G	G	G
8455	G	.	A	A	A
8467	G	.	A	A	A
8476	G	.	A	A	A
8491	T	.	C	C	C
8589	G	.	.	.	A
8680	T	.	.	C	C

8683	T	.	C	C	C
8841	A	.	G	G	G
8895	A	.	.	G	.
8989	C	.	T	T	T
8997	G	.	A	A	A
9022	G	.	A	A	A
9211	C	.	N	N	C
9217	T	.	C	C	C
9309	A	.	G	G	G
9416	T	.	.	C	.
9439	G	.	A	A	A
9449	A	.	G	G	G
9562	A	.	G	G	G
9591	T	.	C	G	C
9596	A	.	G	G	G
9607	G	.	A	A	A
9610	T	.	C	C	C
9639	A	.	G	G	G
9917	C	.	T	T	T
9940	T	.	C	C	C
9971	A	.	C	C	C
9977	A	.	G	G	G
10004	T	.	.	C	C
10025	A	.	G	.	.
10031	A	.	G	G	G
10049	G	.	A	A	A
10127	T	.	C	C	C
10130	G	.	.	A	.
10202	C	.	T	T	T
10277	C	.	.	T	.
10355	A	.	.	G	.
10424	T	.	.	C	.
10569	C	.	T	T	T
10629	C	.	T	T	T
10638	G	.	.	A	A
10701	A	.	T	T	T
10707	A	.	G	G	G
10749	C	.	T	T	T
10784	A	.	G	G	G

10785	G	.	A	A	A
10800	T	.	C	C	C
10830	G	.	A	A	A
10836	C	.	T	T	T
10881	A	.	G	G	G
10908	C	.	T	T	T
10923	G	.	A	A	A
10989	A	.	G	G	G
11016	A	.	G	G	G
11049	T	.	.	C	C
11073	A	.	G	G	G
11154	A	.	.	G	.
11183	T	.	C	C	C
11193	G	.	A	A	A
11232	C	.	C	C	C
11355	T	.	C	C	C
11382	T	.	C	C	C
11541	C	.	C	C	C
11552	G	.	A	A	A
11598	G	.	A	.	A
11650	T	.	C	C	C
11670	A	.	G	.	G
11739	C	.	T	T	T
11833	A	.	G	G	G
11897	A	.	G	G	G
11915	C	.	T	T	T
11999	A	.	G	G	G
12011	A	.	G	G	G
12020	A	.	G	G	G
12038	C	.	T	T	T
12071	T	.	C	C	C
12080	A	.	G	G	G
12161	T	.	C	C	C
12392	C	.	T	T	T
12422	A	.	G	G	G
12476	G	.	A	A	A
12494	G	.	A	A	A
12521	C	.	T	T	T
12533	T	.	.	C	.

12536	T	.	C	C	C
12548	G	.	A	A	A
12587	G	.	A	A	A
12665	A	.	G	G	G
12667	C	.	.	T	T
12687	T	.	C	C	C
12707	G	.	A	A	A
12734	C	.	.	C	.
12785	G	.	A	A	A
12810	T	.	N	N	N
12868	A	.	G	G	G
12912	C	.	T	T	T
12969	C	.	T	T	T
13002	G	.	A	A	A
13095	T	.	C	C	C
13098	A	.	G	G	G
13185	T	.	C	C	C
13194	C	.	T	T	T
13202	A	.	G	G	G
13221	G	.	.	.	A
13245	G	.	.	.	A
13287	C	.	.	.	T
13298	A	.	.	G	.
13368	G	.	.	A	.
13422	G	.	A	A	A
13427	G	.	A	A	A
13431	G	.	A	A	A
13437	G	.	A	A	A
13467	C	.	T	T	T
13497	G	.	A	A	A
13545	G	.	C	C	C
13554	A	.	G	G	G
13596	T	.	.	C	C
13662	T	.	C	C	C
13731	A	.	G	G	G
13737	C	.	A	A	A
13761	A	.	G	G	G
13776	A	.	G	G	G
13793	A	.	G	G	G

13794	G	.	A	A	A
13806	T	.	C	C	C
13860	A	.	G	G	G
13877	A	.	G	.	.
13878	T	.	C	C	C
13889	G	.	A	A	A
13946	G	.	.	.	A
14054	G	.	.	A	A
14073	G	.	A	A	A
14097	A	.	G	G	G
14184	A	.	G	G	G
14226	C	.	T	T	T
14240	A	.	G	G	G
14247	A	.	.	.	G
14310	A	.	G	G	G
14361	T	.	.	C	.
14575	T	.	.	C	.
14616	A	.	G	G	G
14631	A	.	T	T	T
14636	A	.	G	G	G
14682	G	.	C	C	C
14692	A	.	G	G	G
14706	A	.	G	G	G
14710	T	.	.	.	C
14725	A	.	G	G	G
14729	A	.	G	G	G
14752	T	.	C	C	C
14806	G	.	A	A	A
14812	T	.	C	C	C
14825	A	.	G	G	G
14839	G	.	A	A	A
14850	A	.	G	G	G
14879	A	.	N	N	N
14898	A	.	G	G	G
14899	T	.	G	G	C
14908	A	.	G	G	G
14959	G	.	A	A	A
14991	C	.	.	T	T
15023	A	.	G	G	G

15025	A	.	G	G	G
15117	T	.	.	C	.
15171	A	.	G	G	G
15182	A	.	G	G	G
15188	A	.	C	C	C
15195	A	.	G	G	G
15312	T	.	C	C	C
15385	A	.	.	.	G
15412	A	.	.	G	G
15450	G	.	.	A	.
15452	T	.	C	C	C
15458	T	.	C	C	C
15482	C	.	.	T	T
15484	G	.	A	A	A
15497	A	.	G	G	G
15533	T	.	C	C	C
15569	G	.	A	A	.
15688	G	.	A	A	A
15695	C	.	G	G	G
15803	G	.	A	A	A
15813	A	.	G	G	G
15927	C	.	T	T	T
15951	T	.	.	C	C
15981	A	.	G	G	G
15987	T	.	C	C	C
16050	C	.	T	T	T
16056	T	.	C	C	C
16089	T	.	C	C	C
16137	G	.	A	A	A
16139	A	.	G	.	.
16194	A	.	G	G	G
16196	T	.	.	C	.
16290	A	.	T	T	T
16293	G	.	.	.	A
16341	T	.	C	C	C
16344	G	.	T	T	T

Table S10: *Eriocheir japonica*

Sampling site	Country	Latitude	Longitude	Coordinate source	Collection date	GenBank accession	BOLD accession	n	n haplo-types	Reference	Notes
Tokushima	Japan	34.088893	134.562378	Coordinates inferred with google maps from location mentioned in publication	NA	AF317330, AF317331	GBCMD0085-06, GBCMD0086-06	2	2	Tang et al., 2003	
Shimonoseki	Japan	33.946813	130.91658	Coordinates inferred with google maps from location mentioned in publication	NA	AY640095, AY640096, AY640097, AY640098, AY640099, AY640100, FJ750312, FJ750313, FJ750314, FJ750315, FJ750316	GBCMD0409-06, GBCMD0410-06, GBCMD0411-06, GBCMD0412-06, GBCMD0413-06, GBCMD0414-06, GBCMD3667-09, GBCMD3666-09, GBCMD3665-09, GBCMD3664-09, GBCMD3663-09	12	11	Xu et al., 2009	
Chiba	Japan	35.599893	140.107342	Coordinates inferred with google maps from location mentioned in publication	NA	AY640101, FJ750312, FJ750317	GBCMD0415-06, BCMD3667-09, GBCMD3662-09	8	3	Xu et al., 2009	
Sumjin	Korea	35.564722	127.782142	Coordinates inferred with google maps from location mentioned in publication	NA	AY640101, FJ750318, FJ750319	GBCMD0415-06, GBCMD3661-09, GBCMD3660-09	8	3	Xu et al., 2009	
Vladivostok	Russia	43.118493	131.877268	Coordinates inferred with google maps from location mentioned in publication	NA	AY640101, AY640102	GBCMD0415-06, GBCMD0416-06	3	1	Xu et al., 2009	
Rolandswerth	Germany	50.645000	7.208	Coordinates available in GenBank record	2009	KT208530	BNSDE069-11	1	1	Raupach et al. 2015	Deposited as *Eriocheir sinensis*
NA	Holland	52.374865	4.488154	Coordinates inferred with google maps from location mentioned in BOLD	2011	NA	CBCC038-11, CBCC039-11, CBCC040-11	3	1	BOLD	Deposited as *Eriocheir sinensis*
Vistula River	Poland	54.34	19.53	Coordinates available in BOLD	2015	NA	OZIMP066-15	1	1	BOLD	Deposited as *Eriocheir sinensis*

Table S11: *Eriocheir sinensis*

Sampling site	Country	range	Latitude	Longitude	Coordinate source	Collection date	GenBank accession	BOLD accession	n	n haplotypes	Reference
Changjiang, Yangtze	China	native	29.220565	117.11744	Coordinates available in publication	NA	AF516702	GBCMD0175-06	4	1	Chu et al., 2003
Dalian City	China	native	38.940739	121.623672	Coordinates inferred with google maps from location mentioned in publication	NA	KP064329, KP126617	GBCMA10368-15, GBCMA10369-15	2	2	Li et al., 2016
Feiyunjiang	China	native	27.775756	120.621054	Coordinates inferred with google maps from location mentioned in publication	NA	AY640082, AY640083, AY640085	GBCMD0396-06, GBCMD0397-06, GBCMD0399-06	10	3	Xu et al., 2009
Hangzhou	China	native	30.166667	120.583333	Coordinates available in publication	1999 to 2000	AF435113, AF435114, AF435117	GBCMD0123-06, GBCMD0124-06, GBCMD0127-06	6	3	Hanfling et al., 2002
Liahoe	China	native	41.376010	122.643126	Coordinates available in publication	1999 to 2000	AF435113, AF435114, AF435115, AY640082, FJ750306, FJ750307, FJ750308, FJ750309	GBCMD0123-06, GBCMD0124-06, GBCMD0125-06, GBCMD0396-06, GBCMD3673-09, GBCMD3672-09, GBCMD3671-09, GBCMD3670-09	16	6	Hanfling et al., 2002
Nantong, Yangtze	China	native	31.966667	120.75	Coordinates inferred with google maps from location mentioned in publication	1999 to 2000	AF435113, AF435114, AF435117, AF435119	GBCMD0123-06, GBCMD0124-06, GBCMD0127-06, GBCMD0129-06	10	4	Hanfling et al., 2002
Oujiang	China	native	27.977874	120.770467	Coordinates inferred with google maps from location mentioned in publication	NA	AY640082	GBCMD0396-06	11	1	Xu et al., 2009
Tongan	China	native	24.722747	118.152148	Coordinates inferred with google maps from location mentioned in publication	NA	AY640082	GBCMD0396-06	8	1	Xu et al., 2009
Wuhu, Anhui	China	native	31.352859	118.432941	Coordinates available in publication	NA	AY274302, NC006992	GBMNA12372-19, NA	2	1	Sun et al., 2005
Yangtze	China	native	31.514143	121.461574	Sampling site available in GenBank record, coordinates inferred with google maps	NA	DQ438943, KY041629	GBCMD0864-06, NA	2	2	NA
Zhenjiang, Yangtze	China	native	32.257417	119.456195	Coordinates inferred with google maps from location mentioned in publication	NA	AY640082, AY640083	GBCMD0396-06, GBCMD0397-06	8	2	Wang et al., 2008
Gaochun, Jiangsu	China	native	31.29332	118.933018	Coordinates inferred with google maps from location mentioned in publication	NA	AF317333	GBCMD0088-06	1	1	Tang et al., 2003

Panjin, Liaoning	China	native	41.136480	122.075468	Coordinates inferred with google maps from location mentioned in publication	NA	AF317335	GBCMD0089-06	1	1	Tang et al., 2003
Shandong	China	native	36.095087	120.288494	Coordinates inferred with google maps from location mentioned in publication	2003 to 2008	AF279269	GBCMD0059-06	1	1	Czerniejewski et al., 2012
Yancheng	China	native	33.351046	120.176321	Coordinates inferred with google maps from location mentioned in publication	NA	KM516908	GBMNA16953-19	1	1	Liu et al., 2015
Yueqing, Zhejiang	China	native	28.126682	121.063465	Coordinates inferred with google maps from location mentioned in publication	NA	AF317337	NA	1	1	Tang et al., 2003
Vladivostok	Russia	native	43.118493	131.877268	Coordinates inferred with google maps from location mentioned in publication	NA	AY640086, AY640087	GBCMD0400-06, GBCMD0401-06	10	2	Xu et al., 2009
Geumgang	South Korea	native	35.995000	126,73324	Coordinates inferred with google maps from location mentioned in publication	NA	AY640084, FJ750310, FJ750311	GBCMD0398-06, GBCMD3669-09, GBCMD3668-09	15	2	Xu et al., 2009
Yongkang	Taiwan	native	23.100391	120.192711	Coordinates inferred with google maps from location mentioned in publication	2003 to 2008	AF105248	GBCMD0047-06	1	1	Czerniejewski et al., 2012
Thames	England	introduced	51.450000	0.416667	Coordinates available in publication	1999 to 2000	AF435113, AF435116, AF435117, AF435118	GBCMD0123-06, GBCDA2824-12, GBCMD0127-06, GBCMD0128-06	15	4	Hanfling et al., 2002
Aukrug	Germany	introduced	54.083333	9.816667	Coordinates available in publication	2008 to 2010	JQ956428, JQ956430	GBCMD12740-13, GBCMD12738-13	16	2	Otto 2012
Eckernfoerde	Germany	introduced	54.467649	9.841365	Coordinates inferred with google maps from location mentioned in publication	2008 to 2010	AF435113, JQ956429	GBCMD0123-06, GBCMD12739-13	18	2	Otto 2012
Eider	Germany	introduced	54.316667	9.133333	Coordinates available in publication	2008 to 2010	AF435113, JQ956426, JQ956429, JQ956430, JQ956431	GBCMD0123-06, GBCMD12742-13, GBCMD12739-13, GBCMD12738-13, GBCMD12737-13	45	5	Otto 2012
Finkenwerder, Elbe	Germany	introduced	53.548473	9.834498	Coordinates inferred with google maps from location mentioned in publication	2008 to 2010	AF435113, AF435117, JQ956428, JQ956430	GBCMD0123-06, GBCMD0127-06, GBCMD12740-13, GBCMD12738-13	40	4	Otto 2012

Flemhude	Germany	introduced	54.340556	9.964444	Coordinates available in publication	2008 to 2010	AF435113, JQ956425, JQ956427, JQ956429, JQ956430, JQ956431	GBCMD0123-06, GBCMD12743-13, GBCMD12741-13, GBCMD12739-13, GBCMD12738-13, GBCMD12737-13	53	6	Otto 2012
Hemmelsdorf	Germany	introduced	53.966667	10.8	Coordinates available in publication	2008 to 2010	AF435113	GBCMD0123-06	10	1	Otto 2012
Laascher See	Germany	introduced	53.033333	11.416667	Coordinates available in publication	1999 to 2000	AF435113, AF435116, AF435117	GBCMD0123-06, GBCDA2824-12, GBCMD0127-06	15	3	Hanfling et al., 2002
NOK	Germany	introduced	54.367000	10.1	Coordinates available in publication	2003 to 2014	KT208443	BNSDE258-12, BNSDE259-12	2	1	Raupach et al. 2015
Oldenburg, Weser	Germany	introduced	53.100000	8.15	Coordinates available in publication	1999 to 2000	AF435113, AF435115, AF435116, AF435117	GBCMD0123-06, GBCMD0125-06, GBCDA2824-12, GBCMD0127-06	14	4	Hanfling et al., 2002
Osterholz, Elbe	Germany	introduced	52.750000	12.033333	Coordinates available in publication	1999 to 2000	AF435113, AF435116, AF435117	GBCMD0123-06, GBCDA2824-12, GBCMD0127-06	15	3	Hanfling et al., 2002
Schlei	Germany	introduced	54.595976	9.852501	Coordinates inferred with google maps from location mentioned in publication	2008 to 2010	JQ956427, JQ956429, JQ956431	GBCMD12741-13, GBCMD12739-13, GBCMD12737-13	11	3	Otto 2012
Soholmer Au	Germany	introduced	54.700000	8.866667	Coordinates available in publication	2008 to 2010	JQ956429, JQ956430, JQ956431	GBCMD12739-13, GBCMD12738-13, GBCMD12737-13	36	3	Otto 2012
Brandenburg, Elbe	Germany	introduced	52.398583	12.528806	Coordinates available in publication	2003 to 2008	HM640255	GBCMA7168-14	1	1	Czerniejewski et al. 2012
Bremen	Germany	introduced	53.545000	8.57	Coordinates available in GenBank record	2003 to 2014	KT209097	BNSC602-15	1	1	Raupach et al. 2015
Jadebusen	Germany	introduced	53.326000	7.245	Coordinates available in GenBank record	2003 to 2014	KT208532	BNSDE368-14	1	1	Raupach et al. 2015
NA	Netherlands	introduced	52.374865	4.488154	BOLD	23.03.2011	NA	CBCC037-11	1	1	BOLD
Odra River	Poland	introduced	53.289786	14.493875	Coordinates inferred with google maps from location mentioned in publication	2003 to 2008	HM640254, HQ534048	GBCMA7619-14, GBCDA2823-12	2	1	Czerniejewski et al. 2012
Vistula	Poland	introduced	54.272242	18.944731	Coordinates inferred with google maps from location mentioned in publication	2003 to 2008	HM640253, HQ534046, HQ534047	GBCMA7620-14, GBCDA2821-12, GBCDA2822-12, OZIMP065-15	4	2	Czerniejewski et al. 2012; BOLD

Baltic Sea	Poland	introduced	54.622652	16.797764	Coordinates inferred with google maps from location mentioned in publication	NA	DQ882062	FCDPA074-04	1	1	BOLD
Tagus	Portugal	introduced	38.883333	−9.016667	Coordinates available in publication	1999 to 2000	AF435113, AF435116, AF435117	GBCMD0123-06, GBCDA2824-12, GBCMD0127-06	16	3	Hanfling et al., 2002
Kattegat	Sweden	introduced	57.687	11.841	BOLD	12.08.2003	NA	SWEMA461-15|MG935182	1	1	BOLD
Lake Vanern	Sweden	introduced	59.031960	13.625511	Coordinates inferred with google maps from location mentioned in publication	2003 to 2008	HM640257	GBCMA7727-14	1	1	Czerniejewski et al., 2012
Sacramento	USA	introduced	38.116667	−121.683333	Coordinates inferred with google maps from location mentioned in publication	1999 to 2000	AF435116	GBCDA2824-12	7	1	Hanfling et al., 2002
San Francisco	USA	introduced	37.650000	−122.366667	Coordinates inferred with google maps from location mentioned in publication	1999 to 2000	AF435116, HM640256, HQ534049	GBCDA2824-12, GBCMA7850-14, GBCDA2824-12	18	1	Hanfling et al., 2002
Patapsco River	USA	introduced	39.210349	−76.453416	Chesapeake Bay Barcode Initiative	22.06.2009	MH087510	NA	1	1	Chesapeake Bay Barcode Initiative (https://serc.si.edu/projects/species-diversity-chesapeake-bay)

Table S12: pairwise population

Site	China: Feiyunjiang	China: Hangzhou	China: Liaohe	China: Nantong, Yangtze	China: Oujiang	China: Tongan	China: Zhenjiang, Yangtze	Russia: Vladivostok	South Korea: Geumgang	England: Thames	Germany: Aukrug	Germany: Eckernfoerde	Germany: Eider	Germany: Finkenwerder, Elbe	Germany: Flemhude	Germany: Hemmelsdorf	Germany: Laascher See	Germany: Oldenburg, Weser	Germany: Osterholz, Elbe	Germany: Schlei	Germany: Soholmer Au	Portugal: Tagus	USA: Sacramento	USA: San Francisco
China: Feiyunjiang		0,118	0,144	0,099	0,266	0,214	0,032	0,419	0,689	−0,021	0,589	0,290	0,076	0,238	0,075	0,250	−0,065	−0,084	−0,020	0,199	0,224	0,007	0,518	0,666
China: Hangzhou	0,026		−0,068	−0,105	0,259	0,192	0,078	0,428	0,498	0,139	0,604	0,275	0,126	0,294	0,123	0,239	0,172	0,121	0,150	0,205	0,298	0,130	0,698	0,828
China: Liaohe	0,031	0,000		0,002	0,241	0,202	0,153	0,416	0,329	0,183	0,582	0,292	0,206	0,334	0,206	0,229	0,188	0,149	0,188	0,281	0,354	0,178	0,540	0,654
China: Nantong, Yangtze	0,023	0,000	0,005		0,220	0,171	0,021	0,395	0,432	0,089	0,514	0,274	0,124	0,224	0,104	0,205	0,131	0,097	0,093	0,213	0,259	0,138	0,551	0,693
China: Oujiang	0,020	0,020	0,036	0,029		NA	0,346	0,624	0,928	0,300	0,782	0,177	0,179	0,434	0,180	NA	0,321	0,232	0,321	0,328	0,404	0,054	1,000	1,000
China: Tongan	0,020	0,020	0,035	0,028	0,000		0,286	0,578	0,918	0,258	0,758	0,142	0,157	0,412	0,159	NA	0,279	0,190	0,279	0,278	0,380	0,023	1,000	1,000
China: Zhenjiang, Yangtze	0,010	0,020	0,035	0,011	0,022	0,022		0,429	0,736	−0,045	0,542	0,331	0,042	0,132	−0,016	0,328	0,035	−0,007	−0,045	0,206	0,185	0,083	0,716	0,828
Russia: Vladivostok	0,103	0,134	0,124	0,127	0,127	0,126	0,107		0,755	0,406	0,647	0,629	0,360	0,460	0,405	0,610	0,419	0,429	0,419	0,465	0,434	0,527	0,643	0,765
South Korea: Geumgang	0,108	0,050	0,047	0,065	0,083	0,083	0,109	0,215		0,633	0,841	0,784	0,420	0,610	0,471	0,925	0,652	0,660	0,652	0,619	0,557	0,712	0,970	0,980
England: Thames	0,003	0,033	0,040	0,021	0,036	0,036	0,001	0,104	0,126		0,479	0,339	0,066	0,114	0,034	0,287	−0,040	−0,031	−0,068	0,230	0,163	0,111	0,454	0,582
Germany: Aukrug	0,160	0,194	0,200	0,159	0,224	0,223	0,128	0,241	0,311	0,118		0,772	0,299	0,165	0,350	0,775	0,533	0,578	0,495	0,604	0,218	0,690	0,728	0,807
Germany: Eckernfoerde	0,027	0,027	0,043	0,036	0,006	0,006	0,029	0,136	0,091	0,044	0,234		0,189	0,466	0,192	0,167	0,354	0,275	0,354	0,225	0,415	0,154	0,867	0,907
Germany: Eider	0,029	0,053	0,071	0,044	0,054	0,053	0,022	0,147	0,149	0,024	0,105	0,052		0,132	0,017	0,173	0,085	0,081	0,066	0,065	0,068	0,128	0,335	0,403
Germany: Finkenwerder, Elbe	0,056	0,081	0,091	0,055	0,105	0,104	0,031	0,146	0,196	0,026	0,034	0,114	0,039		0,105	0,428	0,186	0,219	0,121	0,376	0,111	0,343	0,455	0,524

Germany: Flemhude	0,020	0,036	0,051	0,025	0,038	0,037	0,005	0,124	0,129	0,010	0,095	0,038	0,007	0,024		0,174	0,080	0,061	0,033	0,151	0,137	0,113	0,439	0,498
Germany: Hemmels-dorf	0,020	0,020	0,035	0,028	0,000	0,000	0,022	0,127	0,083	0,036	0,224	0,006	0,054	0,105	0,038		0,308	0,219	0,308	0,313	0,397	0,045	1,000	1,000
Germany: Laascher See	−0,002	0,036	0,040	0,028	0,036	0,036	0,010	0,103	0,125	−0,001	0,137	0,044	0,029	0,041	0,019	0,036		−0,049	−0,042	0,239	0,191	0,104	0,389	0,521
Germany: Oldenburg, Weser	−0,004	0,024	0,029	0,019	0,020	0,019	0,005	0,099	0,108	0,001	0,150	0,027	0,028	0,048	0,016	0,020	−0,001		−0,032	0,221	0,231	0,013	0,527	0,651
Germany: Osterholz, Elbe	0,003	0,032	0,040	0,020	0,036	0,036	0,001	0,103	0,125	−0,004	0,118	0,044	0,023	0,026	0,010	0,036	−0,001	0,00		0,239	0,165	0,117	0,479	0,604
Germany: Schlei	0,048	0,063	0,081	0,065	0,051	0,050	0,051	0,168	0,142	0,057	0,229	0,031	0,027	0,116	0,040	0,051	0,057	0,048	0,057		0,207	0,255	0,571	0,703
Germany: Soholmer Au	0,077	0,124	0,138	0,098	0,140	0,139	0,065	0,193	0,235	0,052	0,067	0,136	0,025	0,031	0,039	0,140	0,060	0,074	0,051	0,077		0,333	0,328	0,407
Portugal: Tagus	0,004	0,017	0,028	0,020	0,004	0,003	0,010	0,111	0,089	0,015	0,188	0,010	0,037	0,076	0,024	0,004	0,014	0,004	0,015	0,044	0,106		0,739	0,815
USA: Sacramento	0,072	0,168	0,146	0,154	0,167	0,167	0,130	0,179	0,250	0,083	0,201	0,175	0,128	0,127	0,133	0,167	0,059	0,082	0,082	0,165	0,118	0,121		NA
USA: San Francisco	0,073	0,169	0,147	0,155	0,167	0,167	0,131	0,180	0,250	0,084	0,202	0,176	0,131	0,128	0,135	0,167	0,060	0,083	0,084	0,167	0,120	0,122	0,000	

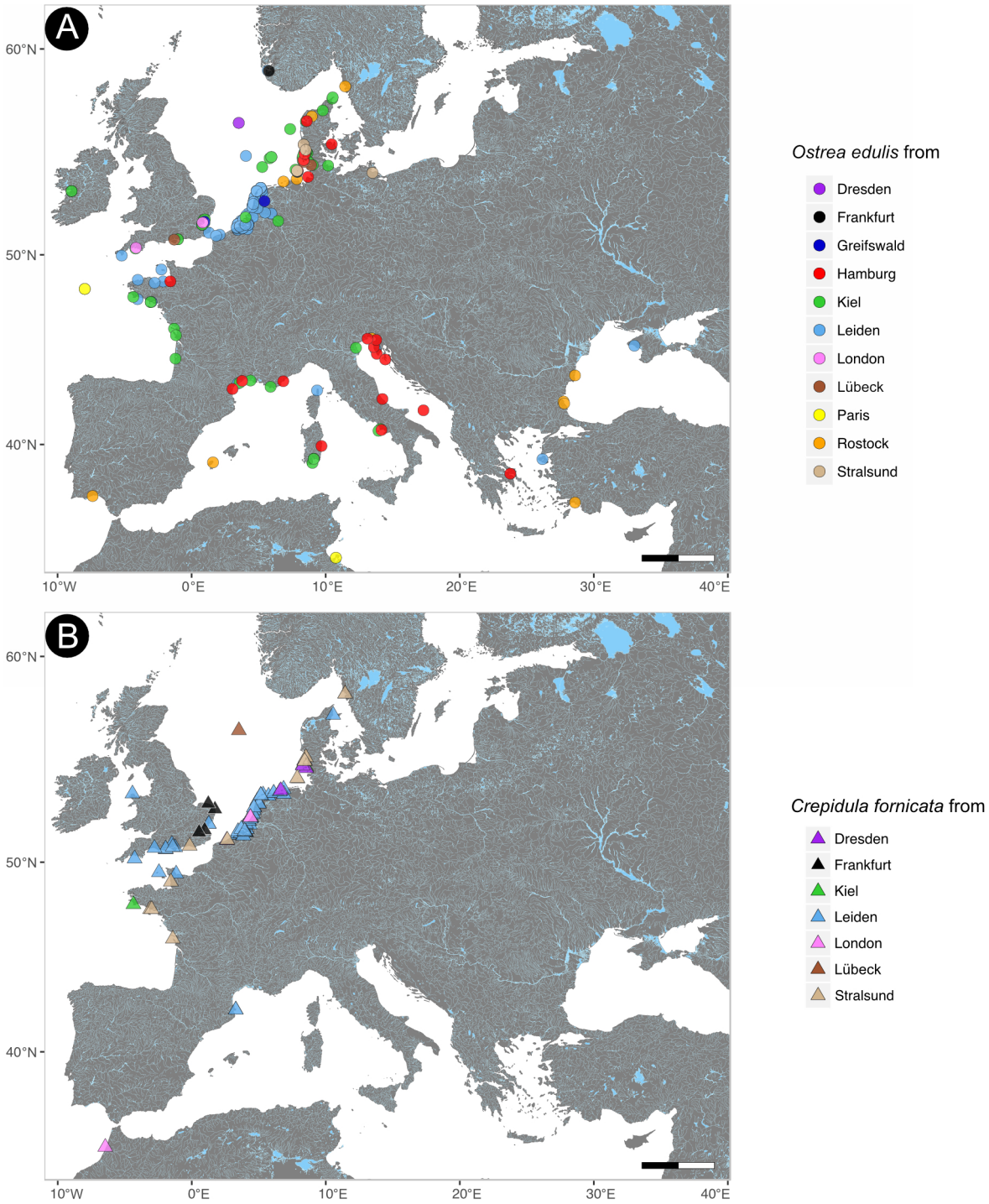

Figure S1: Overview of the distribution of *Ostrea edulis* and *Crepidula fornicata* in Europe based on data of different museum collections across Europe. **(A)** historical distribution of *O. edulis* on one map between the 1820s and 2018 coloured by the museum collection; **(B)** historical distribution of *C. fornicata* on one map between 1926 and 2017 coloured by the museum collection; scale bar = 500km.

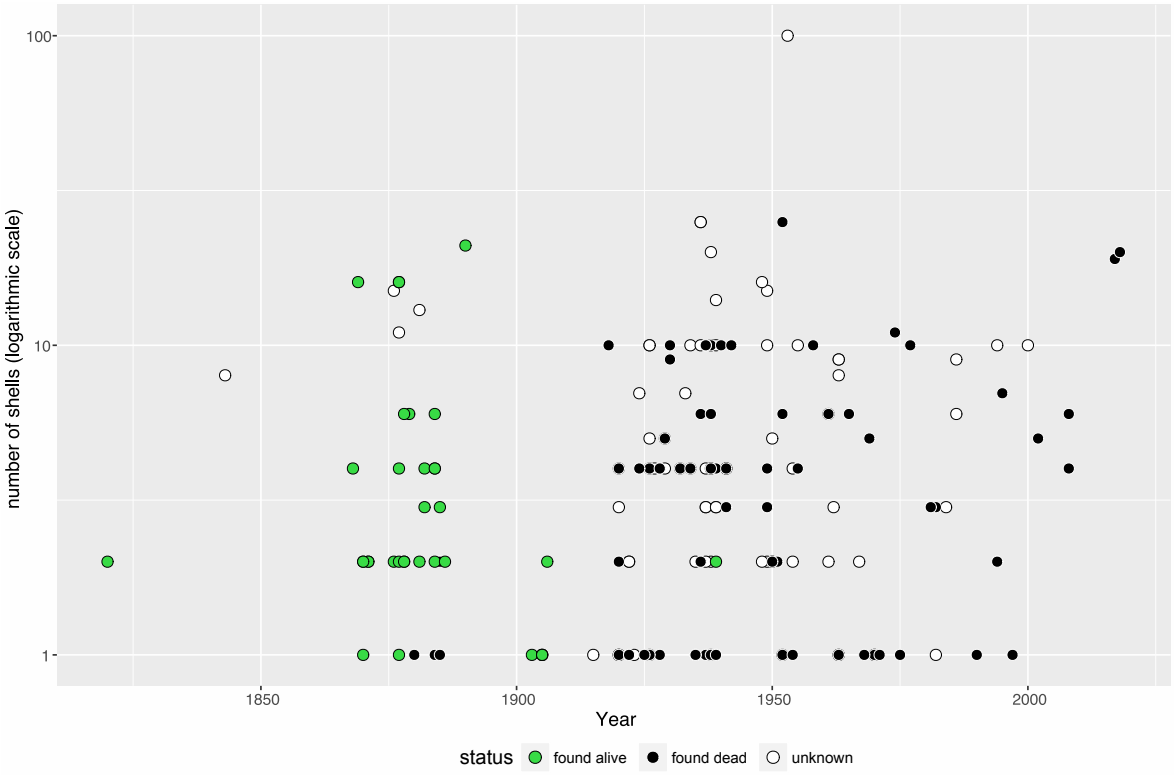

Figure S2: Scatterplot of the number of shells of *O. edulis* from the North Sea housed in natural history collections over time. The values on the y-axis display the number of shells collected annually on a logarithmic scale. The values on the x-axis display the year of sampling.

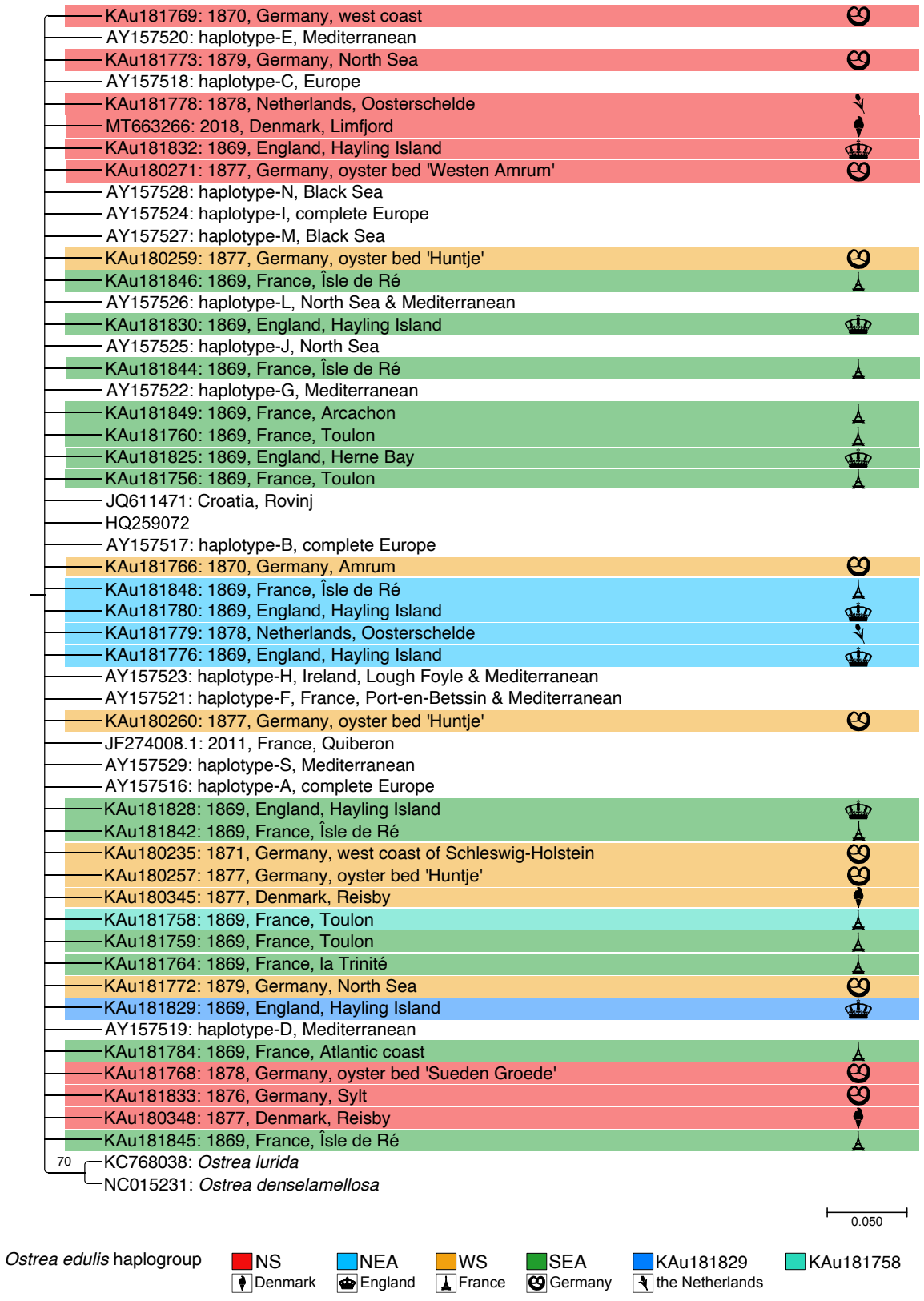

Figure S3: Phylogeny of ancient and modern 12S rRNA sequences mapped against MT663266 (complete mitochondrial genome generated in this study) and controlled with JF274008.1 (complete mitochondrial genome in Genbank) using the maximum likelihood method and General time Reversible model using all sites. Modern DNA was downloaded from Genbank Diaz-Almela et al., 2004; Malkowsky and Klussmann-Kolb, 2012. Bootstrap node support (in percent, from 500 replicates) is shown next to the branches. All branches with less than 35 bootstrap support are collapsed. Colour shading highlights different haplogroups. Phylogeny is rooted with *O. lurida* and *O. denselamellosa*. This analysis involved 54 nucleotide sequences with a total of 280 positions in the final dataset.

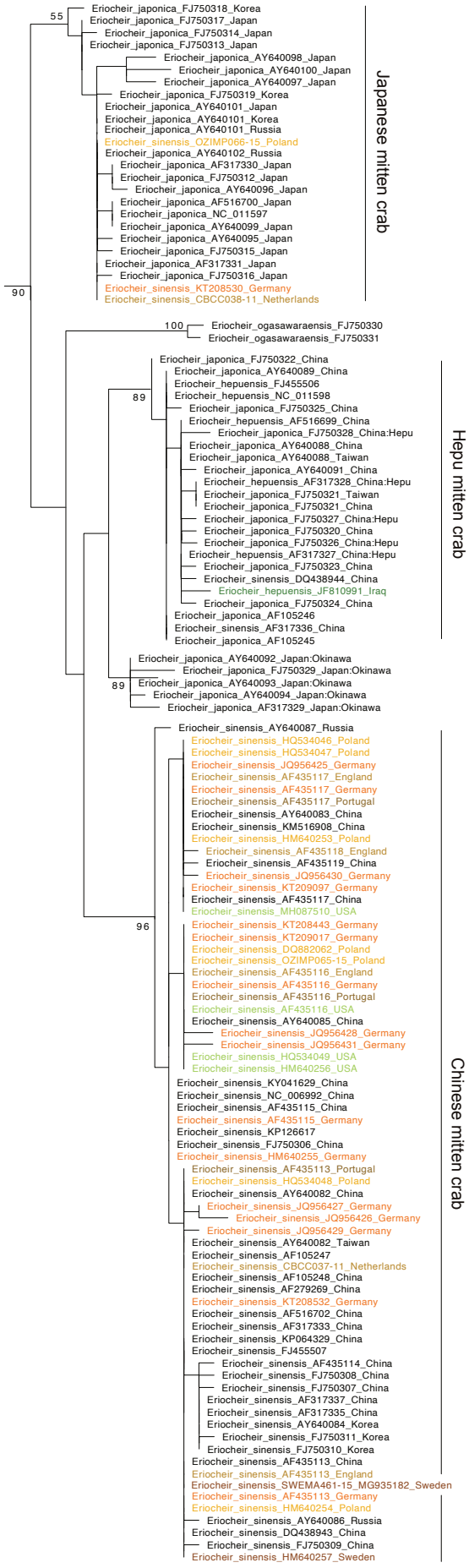

Figure S4: Maximum likelihood tree of mitten crab COI sequences from GenBank and BOLD. Each sequence is labeled with Latin species name as supplied during the sequence submission process, GenBank accession number (or BOLD accession when the respective sequence is not available in GenBank), and country where the crab was collected. Note that the invasive Japanese mitten crab sequences were identified as Chinese mitten crabs (*Eriocheir sinensis*), and that sequences belonging to the monophyletic Hepu mitten crab lineage were submitted as either Hepu mitten crab (*E. hepuensis*), Chinese mitten crab (*E. sinensis*) or Japanese mitten crab (*E. japonica*), reflecting ongoing discussions about their species status. Sequences of individuals from invaded ranges are colored based on the country of collection (yellow to brown: Europe, green: USA and Iraq), while sequences from the native ranges are black. Numbers on branches represent bootstrap support. Branches without numbers have less than 70% bootstrap support. We omitted the outgroups from the figure to save space.

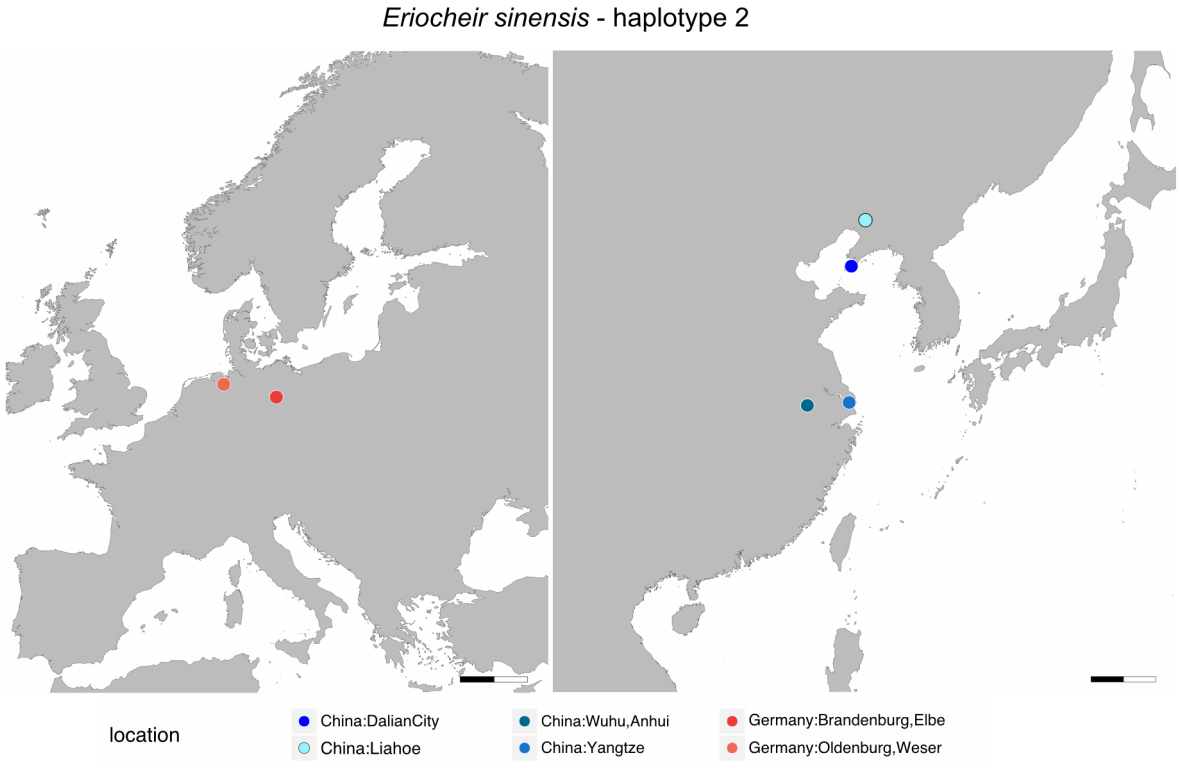

Figure S5: Geographic distribution of COI haplotype H2 of the Chinese mitten crab (*Eriocheir sinensis*).

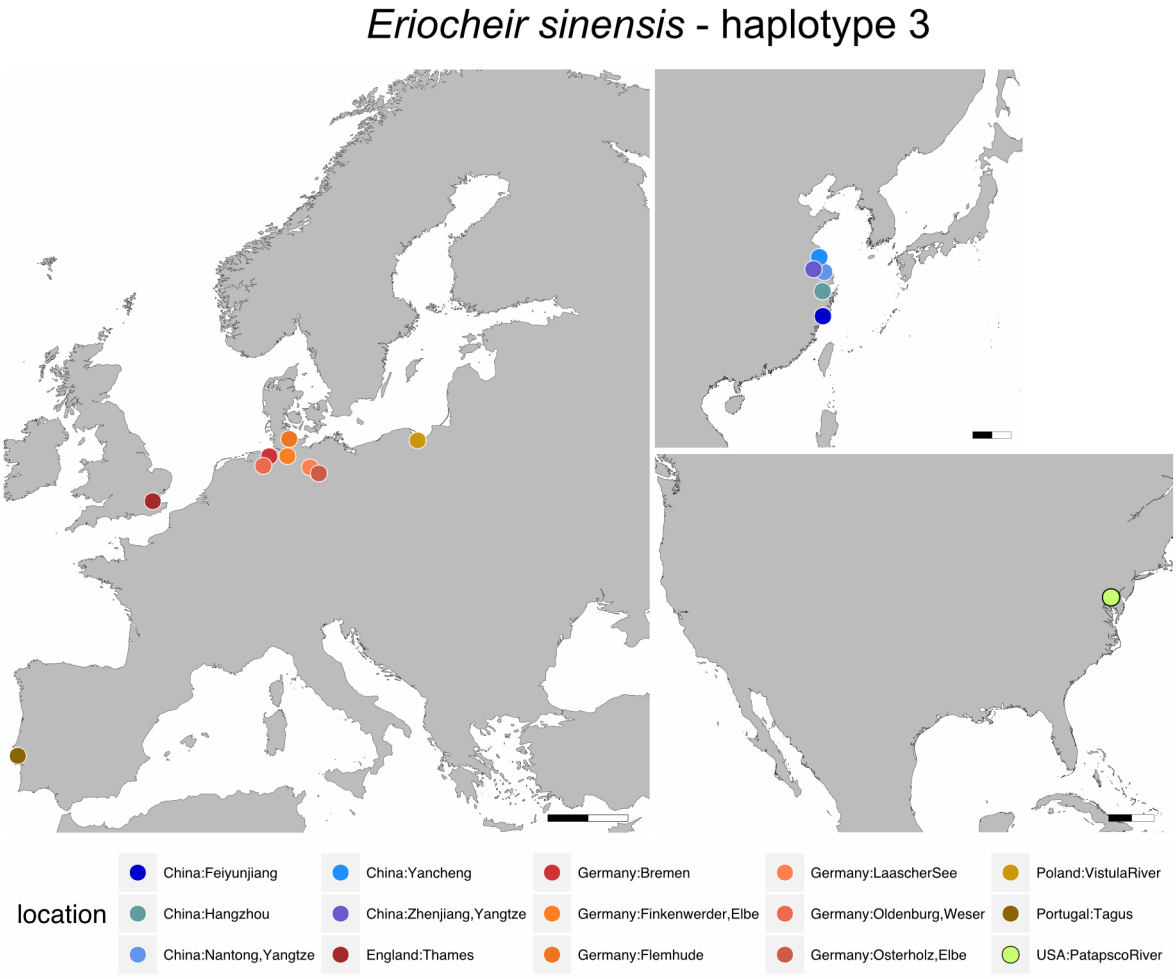

Figure S6: Geographic distribution of COI haplotype H3 of the Chinese mitten crab (*Eriocheir sinensis*).

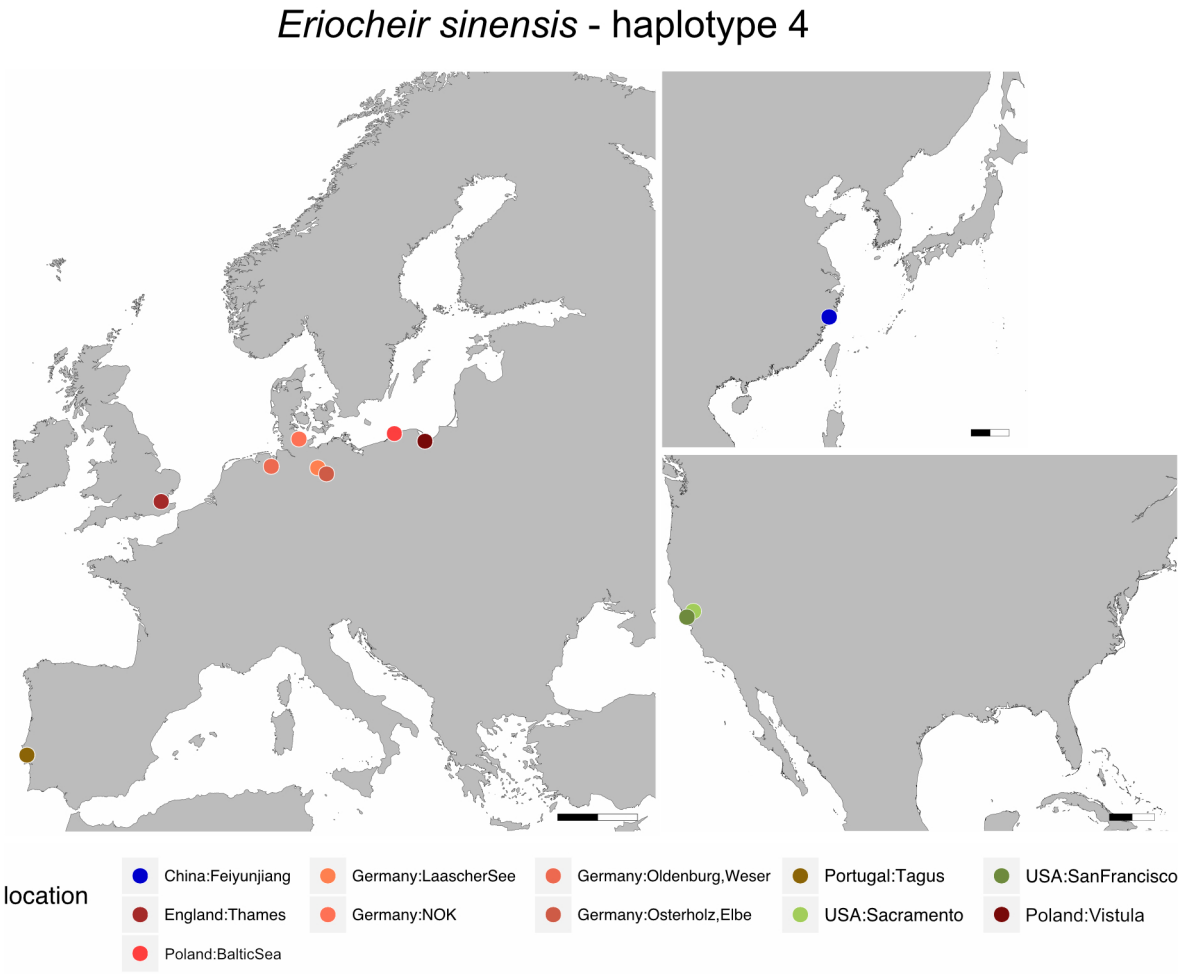

Figure S7: Geographic distribution of COI haplotype H4 of the Chinese mitten crab (*Eriocheir sinensis*).

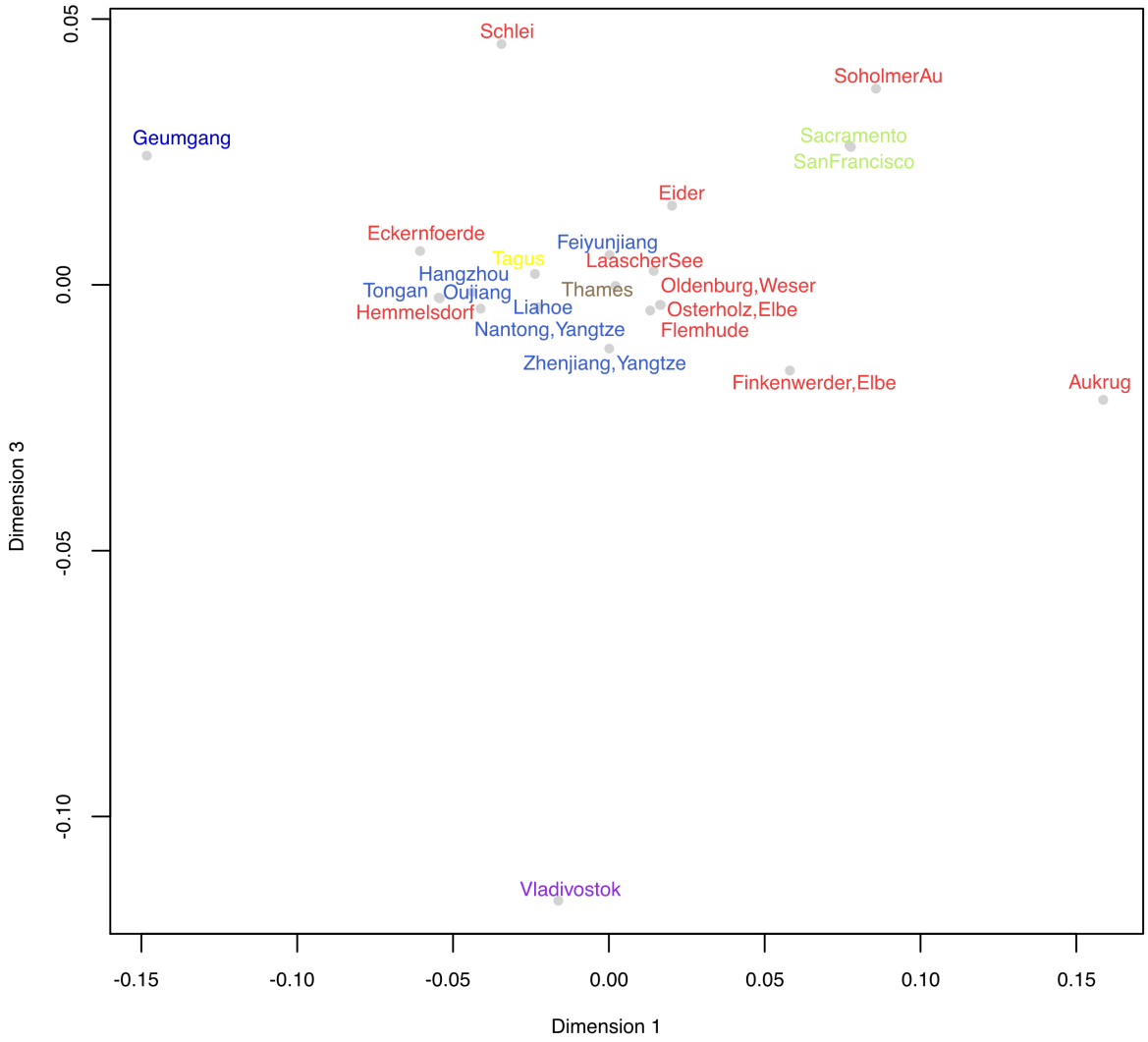

Figure S8: Multidimensional scaling plot of the first and third axes based on Jost's D distances between sampling locations.

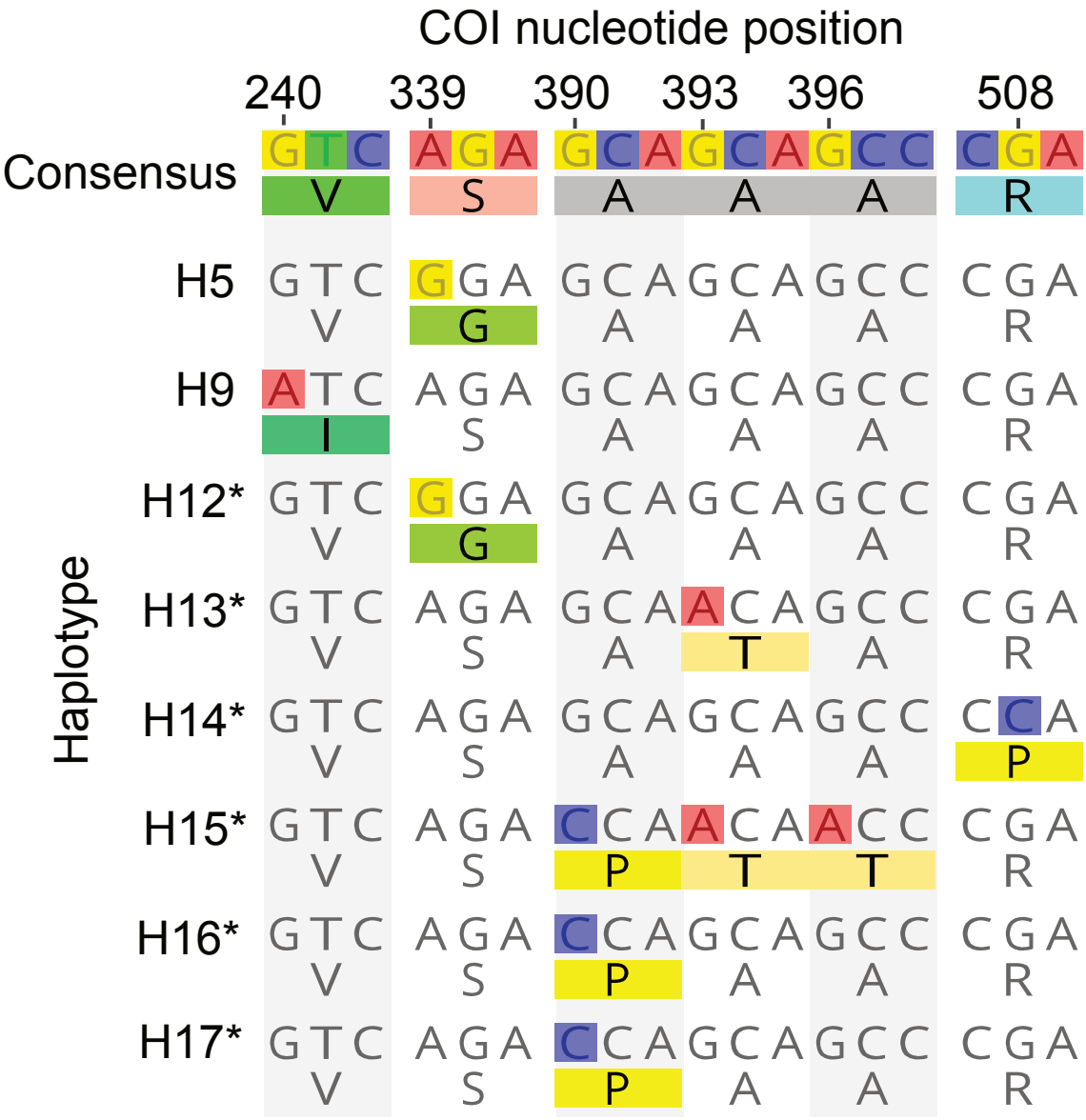

Figure S9: Codons of the COI sequence of Chinese mitten crabs that translate to amino acid substitutions (AAS). Shown are only haplotypes and nucleotide codons with AAS. For each haplotype, the DNA sequence is on top, and the amino acid sequence below. The base numbering refers to the position of the DNA nucleotides relative to the beginning of the COI gene (not the sequenced fragment). Haplotypes with an asterisk were only documented from Northern Germany. Colored amino acids and nucleotides of the haplotypes are likely the derived states.